THERAPEUTIC EPITOPES AND USES THEREOF

The invention relates to epitopes useful in the diagnosis and therapy of coeliac disease, including diagnostics, therapeutics, kits, and methods of using the foregoing.

An immune reaction to gliadin (a component of gluten) in the diet causes coeliac disease. It is known that immune responses in the intestinal tissue preferentially respond to gliadin which has been modified by an intestinal transglutaminase. Coeliac disease is diagnosed by detection of anti-endomysial antibodies, but this requires confirmation by the finding of a lymphocytic inflammation in intestinal biopsies. The taking of such a biopsy is inconvenient for the patient.

Investigators have previously assumed that only intestinal T cell responses provide an accurate indication of the immune response against gliadins. Therefore they have concentrated on the investigation of T cell responses in intestinal tissue[1]. Gliadin epitopes which require transglutaminase modification (before they are recognised by the immune system) are known[2].

The inventors have found the immunodominant T cell A-gliadin epitope recognised by the immune system in coeliac disease, and have shown that this is recognised by T cells in the peripheral blood of individuals with coeliac disease (see WO 01/25793). Such T cells were found to be present at high enough frequencies to be detectable without restimulation (i.e. a 'fresh response' detection system could be used). The epitope was identified using a non-T cell cloning based method which provided a more accurate reflection of the epitopes being recognised. The immunodominant epitope requires transglutaminase modification (causing substitution of a particular glutamine to glutamate) before immune system recognition.

Based on this work the inventors have developed a test which can be used to diagnose coeliac disease at an early stage. The test may be carried out on a sample from peripheral blood and therefore an intestinal biopsy is not required. The test is more sensitive than the antibody tests which are currently being used.

The invention thus provides a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising:

(a) contacting a sample from the host with an agent selected from (i) the epitope comprising sequence which is: SEQ ID NO:1 (PQPELPY)or SEQ ID NO:2 (QLQPFPQPELPYPQPQS), or an equivalent sequence from a naturally occurring homologue of the gliadin represented by SEQ ID NO:3, (ii) an epitope comprising sequence comprising: SEQ ID NO:1, or an equivalent sequence from a naturally occurring homologue of the gliadin represented by SEQ ID NO:3 (shown in Table 1), which epitope is an isolated oligopeptide derived from a gliadin protein, (iii) an analogue of (i) or (ii) which is capable of being recognised by a T cell receptor that recognises (i) or (ii), which in the case of a peptide analogue is not more than 50 amino acids in length, or (iv) a product comprising two or more agents as defined in (i), (ii) or (iii), and (b) determining *in vitro* whether T cells in the sample recognise the agent, recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

Through comprehensive mapping of wheat gliadin T cell epitopes (see Example 13), the inventors have also found epitopes bioactive in coeliac disease in HLA-DQ2+ patients in other wheat gliadins, having similar core sequences (e.g., SEQ ID NOS:18-22) and similar full length sequences (e.g., SEQ ID NOS:31-36), as well as in rye secalins and barley hordeins (e.g., SEQ ID NOS:39-41); see also Tables 20 and 21. Additionally, several epitopes bioactive in coeliac disease in HLA-DQ8+ patients have been identified (e.g., SEQ ID NOS:42-44, 46). This comprehensive mapping thus provides the dominant epitopes recognized by T cells in coeliac patients. Thus, the above-described method and other methods of the invention described herein may be performed using any of these additional identified epitopes, and analogues and equivalents thereof; (i) and (ii) herein include these additional epitopes. That is, the agents of the invention also include these novel epitopes.

The invention also provides use of the agent for the preparation of a diagnostic means for use in a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual, said method comprising

determining whether T cells of the individual recognise the agent, recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

The finding of an immunodominant epitope which is modified by transglutaminase (as well as the additional other epitopes defined herein) also allows diagnosis of coeliac disease based on determining whether other types of immune response to this epitope are present. Thus the invention also provides a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising determining the presence of an antibody that binds to the epitope in a sample from the individual, the presence of the antibody indicating that the individual has, or is susceptible to, coeliac disease.

The invention additionally provides the agent, optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by tolerising T cells which recognise the agent. Also provided is an antagonist of a T cell which has a T cell receptor that recognises (i) or (ii), optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by antagonising such T cells. Additionally provided is the agent or an analogue that binds an antibody (that binds the agent) for use in a method of treating or preventing coeliac disease in an individual by tolerising the individual to prevent the production of such an antibody.

The invention provides a method of determining whether a composition is capable of causing coeliac disease comprising determining whether a protein capable of being modified by a transglutaminase to an oligopeptide sequence as defined above is present in the composition, the presence of the protein indicating that the composition is capable of causing coeliac disease.

The invention also provides a mutant gliadin protein whose wild-type sequence can be modified by a transglutaminase to a sequence that comprises an epitope comprising sequence as defined above, but which mutant gliadin protein has been modified in such a way that it does not contain sequence which can be modified by a transglutaminase to a sequence that comprises such an epitope comprising sequence; or a fragment of such a mutant gliadin protein which is at

least 15 amino acids long and which comprises sequence which has been modified in said way.

The invention also provides a protein that comprises a sequence which is able to bind to a T cell receptor, which T cell receptor recognises the agent, and which sequence is able to cause antagonism of a T cell that carries such a T cell receptor.

Additionally the invention provides a food that comprises the proteins defined above.

SUMMARY OF THE INVENTION

The present invention provides methods of preventing or treating coeliac disease comprising administering to an individual at least one agent selected from: a) a peptide comprising at least one epitope comprising a sequence selected from the group consisting of SEQ ID NOs:18-22, 31-36, 39-44, and 46, and equivalents thereof; and b) an analogue of a) which is capable of being recognised by a T cell receptor that recognises the peptide of a) and which is not more than 50 amino acids in length; and c) optionally, in addition to the agent selected from a) and b), a peptide comprising at least one epitope comprising a sequence selected from SEQ ID NO:1 and SEQ ID NO:2. In some embodiments, the agent is HLA-DQ2-restricted, HLA-DQ8-restricted or one agent is HLA-DQ2-restricted and a second agent is HLA-DQ8-restricted. In some embodiments, the agent comprises a wheat epitope, a rye epitope, a barley epitope or any combination thereof either as a single agent or as multiple agents.

The present invention also provides methods of preventing or treating coeliac disease comprising administering to an individual a pharmaceutical composition comprising an agent above and pharmaceutically acceptable carrier or diluent.

The present invention also provides methods of preventing or treating coeliac disease comprising administering to an individual a pharmaceutical composition comprising an antagonist of a T cell which has a T cell receptor as defined above, and a pharmaceutically acceptable carrier or diluent.

The present invention also provides methods of preventing or treating coeliac disease comprising administering to an individual a composition for tolerising an individual to a gliadin protein to suppress the production of a T cell or antibody response to an agent as defined above, which composition comprises an agent as defined above.

The present invention also provides methods of preventing or treating coeliac disease by 1) diagnosing coeliac disease in an individual by either: a) contacting a sample from the host with at least one agent selected from: i) a peptide comprising at least one epitope comprising a sequence selected from the group consisting of: SEQ ID NOS:18-22, 31-36, 39-44, and 46, and equivalents thereof; and ii) an analogue of i) which is capable of being recognised by a T cell receptor that recognises i) and which is not more than 50 amino acids in length; and iii) optionally, in addition to the agent selected from i) and ii), a peptide comprising at least one epitope comprising a sequence selected from SEQ ID NOS:1 and 2; and determining *in vitro* whether T cells in the sample recognise the agent; recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease; or b) administering an agent as defined above and determining *in vivo* whether T cells in the individual recognise the agent, recognition of the agent indicating that the individual has or is susceptible to coeliac disease; and 2) administering to an individual diagnosed as having, or being susceptible to, coeliac disease a therapeutic agent for preventing or treating coeliac disease.

The present invention also provides agents as defined above, optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by tolerising T cells which recognise the agent.

The present invention also provides antagonists of a T cell which has a T cell receptor as defined above, optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by antagonising such T cells.

The present invention also provides proteins that comprises a sequence which is able to bind to a T cell receptor, which T cell receptor recognises an

agent as defined above, and which sequence is able to cause antagonism of a T cell that carries such a T cell receptor.

The present invention also provides pharmaceutical compositions comprising an agent or antagonist as defined and a pharmaceutically acceptable carrier or diluent.

The present invention also provides compositions for tolerising an individual to a gliadin protein to suppress the production of a T cell or antibody response to an agent as defined above, which composition comprises an agent as defined above.

The present invention also provides compositions for antagonising a T cell response to an agent as defined above, which composition comprises an antagonist as defined above.

The present invention also provides mutant gliadin proteins whose wild-type sequence can be modified by a transglutaminase to a sequence which is an agent as defined in claim 1, which mutant gliadin protein comprises a mutation which prevents its modification by a transglutaminase to a sequence which is an agent as defined above; or a fragment of such a mutant gliadin protein which is at least 15 amino acids long and which comprises the mutation.

The present invention also provides polynucleotides that comprises a coding sequence that encodes a protein or fragment as defined above.

The present invention also provides cells comprising a polynucleotide as defined above or which has been transformed with such a polynucleotide.

The present invention also provides mammals that expresses a T cell receptor as defined above.

The present invention also provides methods of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising: a) contacting a sample from the host with at least one agent selected from i) a peptide comprising at least one epitope comprising a sequence selected from the group consisting of: SEQ ID NOS:18-22, 31-36, 39-44, and 46, and equivalents thereof; and ii) an analogue of i) which is capable of being recognised by a T cell receptor that recognises i) and which is not more than 50 amino acids in length;

and iii) optionally, in addition to the agent selected from i) and ii), a peptide comprising at least one epitope comprising a sequence selected from SEQ ID NOS:1 and 2; and b) determining *in vitro* whether T cells in the sample recognise the agent; recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

The present invention also provides methods of determining whether a composition is capable of causing coeliac disease comprising determining whether a protein capable of being modified by a transglutaminase to an oligopeptide sequence is present in the composition, the presence of the protein indicating that the composition is capable of causing coeliac disease.

The present invention also provides methods of identifying an antagonist of a T cell, which T cell recognises an agent as defined above, comprising contacting a candidate substance with the T cell and detecting whether the substance causes a decrease in the ability of the T cell to undergo an antigen specific response, the detecting of any such decrease in said ability indicating that the substance is an antagonist.

The present invention also provides kits for carrying out any of the method described above comprising an agent as defined above and a means to detect the recognition of the peptide by the T cell.

The present invention also provides methods of identifying a product which is therapeutic for coeliac disease comprising administering a candidate substance to a mammal as defined above which has, or which is susceptible to, coeliac disease and determining whether substance prevents or treats coeliac disease in the mammal, the prevention or treatment of coeliac disease indicating that the substance is a therapeutic product.

The present invention also provides processes for the production of a protein encoded by a coding sequence as defined above which process comprises: a) cultivating a cell described above under conditions that allow the expression of the protein; and optionally b) recovering the expressed protein.

The present invention also provides methods of obtaining a transgenic plant cell comprising transforming a plant cell with a vector as described above to give a transgenic plant cell.

The present invention also provides methods of obtaining a first-generation transgenic plant comprising regenerating a transgenic plant cell transformed with a vector as described above to give a transgenic plant.

The present invention also provides methods of obtaining a transgenic plant seed comprising obtaining a transgenic seed from a transgenic plant obtainable as described above.

The present invention also provides methods of obtaining a transgenic progeny plant comprising obtaining a second-generation transgenic progeny plant from a first-generation transgenic plant obtainable by a method as described above, and optionally obtaining transgenic plants of one or more further generations from the second-generation progeny plant thus obtained.

The present invention also provides transgenic plant cells, plants, plant seeds or progeny plants obtainable by any of the methods described above.

The present invention also provides transgenic plants or plant seeds comprising plant cells as described above.

The present invention also provides transgenic plant cell calluses comprising plant cells as described above obtainable from a transgenic plant cell, first-generation plant, plant seed or progeny as defined above.

The present invention also provides methods of obtaining a crop product comprising harvesting a crop product from a plant according to any method described above and optionally further processing the harvested product.

The present invention also provides food that comprises a protein as defined above.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is illustrated by the accompanying drawings in which:

Figure 1 shows freshly isolated PBMC (peripheral blood mononuclear cell) IFNγ ELISPOT responses (vertical axis shows spot forming cells per 10^6 PBMC) to transglutaminase (tTG)-treated and untreated peptide pool 3 (each peptide 10 μg/ml) including five overlapping 15mers spanning A-gliadin 51-85 (see Table 1) and a-chymotrypsin-digested gliadin (40 μg/ml) in coeliac disease Subject 1, initially in remission following a gluten free diet then challenged with 200g bread daily for three days from day 1 (a). PBMC IFNγ ELISPOT responses by Subject 2 to tTG-treated A-gliadin peptide pools 1-10 spanning the complete A-gliadin protein during ten day bread challenge (b). The horizontal axis shows days after commencing bread.

Figure 2 shows PBMC IFNγ ELISPOT responses to tTG-treated peptide pool 3 (spanning A-gliadin 51-85) in 7 individual coeliac disease subjects (vertical axis shows spot forming cells per 10^6 PBMC), initially in remission on gluten free diet, challenged with bread for three days (days 1 to 3). The horizontal axis shows days after commencing bread. (a). PBMC IFNγ Elispot responses to tTG-treated overlapping 15mer peptides included in pool 3; bars represent the mean (± SEM) response to individual peptides (10 μg/ml) in 6 Coeliac disease subjects on day 6 or 7(b). (In individual subjects, ELISPOT responses to peptides were calculated as a % of response elicited by peptide 12 - as shown by the vertical axis.)

Figure 3 shows PBMC IFNγ ELISPOT responses to tTG-treated truncations of A-gliadin 56-75 (0.1 μM). Bars represent the mean (± SEM) in 5 Coeliac disease subjects. (In individual subjects, responses were calculated as the % of the maximal response elicited by any of the peptides tested.)

Figure 4 shows how the minimal structure of the dominant A-gliadin epitope was mapped using tTG-treated 7-17mer A-gliadin peptides (0.1 μM) including the sequence, PQPQLPY (SEQ ID NO:4) (A-gliadin 62-68) (a), and the same peptides without tTG treatment but with the substitution Q→E65 (b). Each line represents PBMC IFNγ ELISPOT responses in each of three Coeliac disease subjects on day 6 or 7 after bread was ingested on days 1-3. (In individual

subjects, ELISPOT responses were calculated as a % of the response elicited by the 17mer, A-gliadin 57-73.)

Figure 5 shows the amino acids that were deamidated by tTG. A-gliadin 56-75 LQLQPFPQPQLPYPQPQSFP (SEQ ID NO:5) (0.1 μM) was incubated with tTG (50 μg/ml) at 37°C for 2 hours. A single product was identified and purified by reverse phase HPLC. Amino acid analysis allowed % deamidation (Q→E) of each Gln residue in A-gliadin 56-75 attributable to tTG to be calculated (vertical axis).

Figure 6 shows the effect of substituting Q→E in A-gliadin 57-73 at other positions in addition to Q65 using the 17mers: QLQPFPQPELPYPQPES (SEQ ID NO:6) (E57,65), QLQPFPQPELPYPQPES (SEQ ID NO:7) (E65,72), ELQPFPQPELPYPQPES (SEQ ID NO:8) (E57, 65, 72), and QLQPFPQPELPYPQPQS (SEQ ID NO:2) (E65) in three Coeliac disease subjects on day 6 or 7 after bread was ingested on days 1-3. Vertical axis shows % of the E65 response.

Figure 7 shows that tTG treated A-gliadin 56-75 (0.1 μM) elicited IFN-g ELISPOT responses in (a) CD4 and CD8 magnetic bead depleted PBMC. (Bars represent CD4 depleted PBMC responses as a % of CD8 depleted PBMC responses; spot forming cells per million CD8 depleted PBMC were: Subject 4: 29, and Subject 6: 535). (b) PBMC IFNγ ELISPOT responses (spot forming cells/million PBMC) after incubation with monoclonal antibodies to HLA-DR (L243), -DQ (L2) and -DP (B7.21) (10 μg/ml) 1h prior to tTG-treated 56-75 (0.1 μM) in two coeliac disease subjects homozygous for HLA-DQ a1*0501, b1*0201.

Figure 8 shows the effect of substituting Glu at position 65 for other amino acids in the immunodominant epitope. The vertical axis shows the % response in the 3 subjects in relation to the immunodominant epitope.

Figure 9 shows the immunoreactivity of naturally occurring gliadin peptides (measuring responses from 3 subjects) which contain the sequence PQLPY (SEQ ID NO:12) with (shaded) and without (clear) transglutaminase treatment.

Figure 10 shows CD8, CD4, β_7, and α^E -specific immunomagnetic bead depletion of peripheral blood mononuclear cells from two coeliac subjects 6 days after commencing gluten challenge followed by interferon gamma ELISpot. A-gliadin 57-73 QE65 (25mcg/ml), tTG-treated chymotrypsin-digested gliadin (100 mcg/ml) or PPD (10 mcg/ml) were used as antigen.

Figure 11 shows the optimal T cell epitope length.

Figure 12 shows a comparison of A-gliadin 57-73 QE65 with other peptides in a dose response study. On Sheet 12 of 47, Fig. 12(a) discloses the amino acid sequence SEQ ID NO:2. On Sheet 12 of 47, Fig. 12(b) discloses the amino acid sequence SEQ ID NO:101. On Sheet 13 of 47, Fig.12(c) discloses the amino acid sequence SEQ ID NO:10. On Sheet 13 of 47, Fig.12(d) discloses the amino acid sequence SEQ ID NO:72. On Sheet 14 of 47, Fig.12(e) discloses the amino acid sequence SEQ ID NO:13 (labelled E65) and amino acid sequence SEQ ID NO:53 (labelled Q65). On Sheet 15 of 47, Fig.12(f) discloses the amino acid sequence SEQ ID NO:47 (labelled E65) and amino acid sequence SEQ ID NO:102 (labelled Q65). On Sheet 16 of 47, Fig.12(g) discloses the amino acid sequence SEQ ID NO:99 (labelled E) and amino acid sequence SEQ ID NO:44 (labelled Q). On Sheet 17 of 47, Fig.12(h) discloses the amino acid sequence SEQ ID NO:48 (labelled E) and amino acid sequence SEQ ID NO:103 (labelled Q).

Figure 13 shows a comparison of gliadin and A-gliadin 57-73 QE65 specific responses.

Figure 14 shows the bioactivity of gliadin polymorphisms in coeliac subjects. On Sheets 20 through 23 of 47, sixteen amino acids are identified (A-P) in the legend of each figure. The amino acid sequences A through P correspond to the following sequence identifiers:

A – SEQ ID NO: 10

B – SEQ ID NO: 26

C – SEQ ID NO: 51

D – SEQ ID NO: 104

E – SEQ ID NO: 68

F – SEQ ID NO:28

G – SEQ ID NO: 69

H – SEQ ID NO:70

I – SEQ ID NO:71

J – SEQ ID NO:105

K – SEQ ID NO:72

L – SEQ ID NO:73

M – SEQ ID NO:74

N – SEQ ID NO:75

O – SEQ ID NO:97

P – SEQ ID NO:77

Figures 15 and 16 show the defining of the core epitope sequence.

Figures 17 to 27 show the agonist activity of A-gliadin 57-73 QE65 variants. On Sheets 25 through 35 of 47, Figs. 17 through 27 disclose the amino acid sequence SEQ ID NO:2.

Figure 28 shows responses in different patient groups.

Figure 29 shows bioactivity of prolamin homologues of A-gliadin 57-73.

Figure 30 shows, for healthy HLA-DQ2 subjects, the change in IFN-gamma ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 31 shows, for coeliac HLA-DQ2 subjects, the change in IFN-gamma ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 32 shows individual peptide contributions to "summed" gliadin peptide response.

Figure 33 shows, for coeliac HLA-DQ2/8 subject C08, gluten challenge induced IFNγ ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 34 shows, for coeliac HLA-DQ2/8 subject C07, gluten challenge induced IFNγ ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 35 shows, for coeliac HLA-DQ8/7 subject C12, gluten challenge induced IFNγ ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 36 shows, for coeliac HLA-DQ6/8 subject C11, gluten challenge induced IFNγ ELISpot responses to tTG-deamidated gliadin peptide pools.

Detailed Description of the Invention

The term "coeliac disease" encompasses a spectrum of conditions caused by varying degrees of gluten sensitivity, including a severe form characterised by a flat small intestinal mucosa (hyperplastic villous atrophy) and other forms characterised by milder symptoms.

The individual mentioned above (in the context of diagnosis or therapy) is human. They may have coeliac disease (symptomatic or asymptomatic) or be suspected of having it. They may be on a gluten free diet. They may be in an acute phase response (for example they may have coeliac disease, but have only ingested gluten in the last 24 hours before which they had been on a gluten free diet for 14 to 28 days).

The individual may be susceptible to coeliac disease, such as a genetic susceptibility (determined for example by the individual having relatives with coeliac disease or possessing genes which cause predisposition to coeliac disease).

The agent

The agent is typically a peptide, for example of length 7 to 50 amino acids, such as 10 to 40, or 15 to 30 amino acids in length.

SEQ ID NO:1 is PQPELPY. SEQ ID NO:2 is QLQPFPQPELPYPQPQS. SEQ ID NO:3 is shown in Table 1 and is the sequence of a whole A-gliadin. The glutamate at position 4 of SEQ ID NO:1 (equivalent to position 9 of SEQ ID NO:2) is generated by transglutaminase treatment of A-gliadin.

The agent may be the peptide represented by SEQ ID NO:1 or 2 or an epitope comprising sequence that comprises SEQ ID NO:1 which is an isolated oligopeptide derived from a gliadin protein; or an equivalent of these sequences from a naturally occurring gliadin protein which is a homologue of SEQ ID NO:3. Thus the epitope may be a derivative of the protein represented by SEQ ID NO:3. Such a derivative is typically a fragment of the gliadin, or a mutated derivative of the whole protein or fragment. Therefore the epitope of the

invention does not include this naturally occurring whole gliadin protein, and does not include other whole naturally occurring gliadins.

The epitope may thus be a fragment of A-gliadin (e.g. SEQ ID NO:3), which comprises the sequence of SEQ ID NO:1, obtainable by treating (fully or partially) with transglutaminase, i.e. with 1, 2, 3 or more glutamines substituted to glutamates (including the substitution within SEQ ID NO:1).

Such fragments may be or may include the sequences represented by positions 55 to 70, 58 to 73, 61 to 77 of SEQ ID NO:3 shown in Table 1. Typically such fragments will be recognised by T cells to at least the same extent that the peptides represented by SEQ ID NO:1 or 2 are recognised in any of the assays described herein using samples from coeliac disease patients.

Additionally, the agent may be the peptide represented by any of SEQ ID NOS:18-22, 31-36, 39-44, and 46 or a protein comprising a sequence corresponding to any of SEQ ID NOS:18-22, 31-36, 39-44, and 46 (such as fragments of a gliadin comprising any of SEQ ID NOS:18-22, 31-36, 39-44, and 46, for example after the gliadin has been treated with transglutaminase). Bioactive fragments of such sequences are also agents of the invention. Sequences equivalent to any of SEQ ID NOS:18-22, 31-36, 39-44, and 46 or analogues of these sequences are also agents of the invention.

In the case where the epitope comprises a sequence equivalent to the above epitopes (including fragments) from another gliadin protein (e.g. any of the gliadin proteins mentioned herein or any gliadins which cause coeliac disease), such equivalent sequences will correspond to a fragment of a gliadin protein typically treated (partially or fully) with transglutaminase. Such equivalent peptides can be determined by aligning the sequences of other gliadin proteins with the gliadin from which the original epitope derives, such as with SEQ ID NO:3 (for example using any of the programs mentioned herein). Transglutaminase is commercially available (e.g. Sigma T-5398). Table 4 provides a few examples of suitable equivalent sequences.

The agent which is an analogue is capable of being recognised by a TCR which recognises (i) or (ii). Therefore generally when the analogue is added to T

cells in the presence of (i) or (ii), typically also in the presence of an antigen presenting cell (APC) (such as any of the APCs mentioned herein), the analogue inhibits the recognition of (i) or (ii), i.e. the analogue is able to compete with (i) or (ii) in such a system.

The analogue may be one which is capable of binding the TCR which recognises (i) or (ii). Such binding can be tested by standard techniques. Such TCRs can be isolated from T cells which have been shown to recognise (i) or (ii) (e.g. using the method of the invention). Demonstration of the binding of the analogue to the TCRs can then shown by determining whether the TCRs inhibit the binding of the analogue to a substance that binds the analogue, e.g. an antibody to the analogue. Typically the analogue is bound to a class II MHC molecule (e.g. HLA-DQ2) in such an inhibition of binding assay.

Typically the analogue inhibits the binding of (i) or (ii) to a TCR. In this case the amount of (i) or (ii) which can bind the TCR in the presence of the analogue is decreased. This is because the analogue is able to bind the TCR and therefore competes with (i) or (ii) for binding to the TCR.

T cells for use in the above binding experiments can be isolated from patients with coeliac disease, for example with the aid of the method of the invention. Other binding characteristics of the analogue may also be the same as (i) or (ii), and thus typically the analogue binds to the same MHC class II molecule to which the peptide binds (HLA-DQ2 or -DQ8). The analogue typically binds to antibodies specific for (i) or (ii), and thus inhibits binding of (i) or (ii) to such antibodies.

The analogue is typically a peptide. It may have homology with (i) or (ii), typically at least 70% homology, preferably at least 80, 90%, 95%, 97% or 99% homology with (i) or (ii), for example over a region of at least 15 more (such as the entire length of the analogue and/or (i) or (ii), or across the region which contacts the TCR or binds the MHC molecule) contiguous amino acids. Methods of measuring protein homology are well known in the art and it will be understood by those of skill in the art that in the present context, homology is

calculated on the basis of amino acid identity (sometimes referred to as "hard homology").

For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* **12**, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information on the world wide web through the internet at, for example, "www.ncbi.nlm.nih.gov/". This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci.* USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.* USA 90: 5873-5787. One measure of similarity provided by the BLAST

algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The homologous peptide analogues typically differ from (i) or (ii) by 1, 2, 3, 4, 5, 6, 7, 8 or more mutations (which may be substitutions, deletions or insertions). These mutations may be measured across any of the regions mentioned above in relation to calculating homology. The substitutions are preferably 'conservative'. These are defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar – charged	D E
		K R
AROMATIC		H F W Y

Typically the amino acids in the analogue at the equivalent positions to amino acids in (i) or (ii) that contribute to binding the MHC molecule or are responsible for the recognition by the TCR, are the same or are conserved.

Typically the analogue peptide comprises one or more modifications, which may be natural post-translation modifications or artificial modifications. The modification may provide a chemical moiety (typically by substitution of a hydrogen, e.g. of a C-H bond), such as an amino, acetyl, hydroxy or halogen (e.g.

fluorine) group or carbohydrate group. Typically the modification is present on the N or C terminus.

The analogue may comprise one or more non-natural amino acids, for example amino acids with a side chain different from natural amino acids. Generally, the non-natural amino acid will have an N terminus and/or a C terminus. The non-natural amino acid may be an L- or a D- amino acid.

The analogue typically has a shape, size, flexibility or electronic configuration that is substantially similar to (i) or (ii). It is typically a derivative of (i) or (ii). In one embodiment the analogue is a fusion protein comprising the sequence of SEQ ID NO:1 or 2, or any of the other peptides mentioned herein; and non-gliadin sequence.

In one embodiment the analogue is or mimics (i) or (ii) bound to a MHC class II molecule. 2, 3, 4 or more of such complexes may be associated or bound to each other, for example using a biotin/streptavidin based system, in which typically 2, 3 or 4 biotin labelled MHC molecules bind to a streptavidin moiety. This analogue typically inhibits the binding of the (i) or (ii)/MHC Class II complex to a TCR or antibody which is specific for the complex.

The analogue is typically an antibody or a fragment of an antibody, such as a Fab or $(Fab)_2$ fragment. The analogue may be immobilised on a solid support, particularly an analogue that mimics peptide bound to a MHC molecule.

The analogue is typically designed by computational means and then synthesised using methods known in the art. Alternatively the analogue can be selected from a library of compounds. The library may be a combinatorial library or a display library, such as a phage display library. The library of compounds may be expressed in the display library in the form of being bound to a MHC class II molecule, such as HLA-DQ2 or -DQ8. Analogues are generally selected from the library based on their ability to mimic the binding characteristics (i) or (ii). Thus they may be selected based on ability to bind a TCR or antibody which recognises (i) or (ii).

Typically analogues will be recognised by T cells to at least the same extent as any of the agents (i) or (ii), for example at least to the same extent as the

equivalent epitope and preferably to the same extent as the peptide represented by SEQ ID NO:2, is recognised in any of the assays described herein, typically using T cells from coeliac disease patients. Analogues may be recognised to these extents *in vivo* and thus may be able to induce coeliac disease symptoms to at least the same extent as any of the agents mentioned herein (e.g. in a human patient or animal model).

Analogues may be identified in a method comprising determining whether a candidate substance is recognised by a T cell receptor that recognises an epitope of the invention, recognition of the substance indicating that the substance is an analogue. Such TCRs may be any of the TCRs mentioned herein, and may be present on T cells. Any suitable assay mentioned herein can be used to identify the analogue. In one embodiment this method is carried out *in vivo*. As mentioned above preferred analogues are recognised to at least the same extent as the peptide SEQ ID NO:2, and so the method may be used to identify analogues which are recognised to this extent.

In one embodiment the method comprises determining whether a candidate substance is able to inhibit the recognition of an epitope of the invention, inhibition of recognition indicating that the substance is an analogue.

The agent may be a product comprising at least 2, 5, 10 or 20 agents as defined by (i), (ii) or (iii). Typically the composition comprises epitopes of the invention (or equivalent analogues) from different gliadins, such as any of the species or variety of or types of gliadin mentioned herein. Preferred compositions comprise at least one epitope of the invention, or equivalent analogue, from all of the gliadins present in any of the species or variety mentioned herein, or from 2, 3, 4 or more of the species mentioned herein (such as from the panel of species consisting of wheat, rye, barley, oats and triticale). Thus, the agent may be monovalent or multivalent.

Diagnosis

As mentioned above the method of diagnosis of the invention may be based on the detection of T cells that bind the agent or on the detection of antibodies that recognise the agent.

The T cells that recognise the agent in the method (which includes the use mentioned above) are generally T cells that have been pre-sensitised *in vivo* to gliadin. As mentioned above such antigen-experienced T cells have been found to be present in the peripheral blood.

In the method the T cells can be contacted with the agent *in vitro* or *in vivo*, and determining whether the T cells recognise the agent can be performed *in vitro* or *in vivo*. Thus the invention provides the agent for use in a method of diagnosis practiced on the human body. Different agents are provided for simultaneous, separate or sequential use in such a method.

The *in vitro* method is typically carried out in aqueous solution into which the agent is added. The solution will also comprise the T cells (and in certain embodiments the APCs discussed below). The term 'contacting' as used herein includes adding the particular substance to the solution.

Determination of whether the T cells recognise the agent is generally accomplished by detecting a change in the state of the T cells in the presence of the agent or determining whether the T cells bind the agent. The change in state is generally caused by antigen specific functional activity of the T cell after the TCR binds the agent. The change of state may be measured inside (e.g. change in intracellular expression of proteins) or outside (e.g. detection of secreted substances) the T cells.

The change in state of the T cell may be the start of or increase in secretion of a substance from the T cell, such as a cytokine, especially IFN-γ, IL-2 or TNF-α. Determination of IFN-γ secretion is particularly preferred. The substance can typically be detected by allowing it to bind to a specific binding agent and then measuring the presence of the specific binding agent/substance complex. The specific binding agent is typically an antibody, such as polyclonal or monoclonal antibodies. Antibodies to cytokines are commercially available, or can be made using standard techniques.

Typically the specific binding agent is immobilised on a solid support. After the substance is allowed to bind the solid support can optionally be washed to remove material which is not specifically bound to the agent. The agent/substance complex may be detected by using a second binding agent that will bind the complex. Typically the second agent binds the substance at a site which is different from the site which binds the first agent. The second agent is preferably an antibody and is labelled directly or indirectly by a detectable label.

Thus the second agent may be detected by a third agent that is typically labelled directly or indirectly by a detectable label. For example the second agent may comprise a biotin moiety, allowing detection by a third agent which comprises a streptavidin moiety and typically alkaline phosphatase as a detectable label.

In one embodiment the detection system which is used is the *ex-vivo* ELISPOT assay described in WO 98/23960. In that assay IFN-γ secreted from the T cell is bound by a first IFN-γ specific antibody that is immobilised on a solid support. The bound IFN-γ is then detected using a second IFN-γ specific antibody which is labelled with a detectable label. Such a labelled antibody can be obtained from MABTECH (Stockholm, Sweden). Other detectable labels which can be used are discussed below.

The change in state of the T cell that can be measured may be the increase in the uptake of substances by the T cell, such as the uptake of thymidine. The change in state may be an increase in the size of the T cells, or proliferation of the T cells, or a change in cell surface markers on the T cell.

In one embodiment the change of state is detected by measuring the change in the intracellular expression of proteins, for example the increase in intracellular expression of any of the cytokines mentioned above. Such intracellular changes may be detected by contacting the inside of the T cell with a moiety that binds the expressed proteins in a specific manner and which allows sorting of the T cells by flow cytometry.

In one embodiment when binding the TCR the agent is bound to an MHC class II molecule (typically HLA-DQ2 or -DQ8), which is typically present on the

surface of an antigen presenting cell (APC). However as mentioned herein other agents can bind a TCR without the need to also bind an MHC molecule.

Generally the T cells which are contacted in the method are taken from the individual in a blood sample, although other types of samples which contain T cells can be used. The sample may be added directly to the assay or may be processed first. Typically the processing may comprise diluting of the sample, for example with water or buffer. Typically the sample is diluted from 1.5 to 100 fold, for example 2 to 50 or 5 to 10 fold.

The processing may comprise separation of components of the sample. Typically mononuclear cells (MCs) are separated from the samples. The MCs will comprise the T cells and APCs. Thus in the method the APCs present in the separated MCs can present the peptide to the T cells. In another embodiment only T cells, such as only CD4 T cells, can be purified from the sample. PBMCs, MCs and T cells can be separated from the sample using techniques known in the art, such as those described in Lalvani *et al* (1997) *J. Exp. Med.* **186**, p859-865.

In one embodiment, the T cells used in the assay are in the form of unprocessed or diluted samples, or are freshly isolated T cells (such as in the form of freshly isolated MCs or PBMCs) which are used directly *ex vivo*, i.e. they are not cultured before being used in the method. Thus the T cells have not been restimulated in an antigen specific manner *in vitro*. However the T cells can be cultured before use, for example in the presence of one or more of the agents, and generally also exogenous growth promoting cytokines. During culturing the agent(s) are typically present on the surface of APCs, such as the APC used in the method. Pre-culturing of the T cells may lead to an increase in the sensitivity of the method. Thus the T cells can be converted into cell lines, such as short term cell lines (for example as described in Ota *et al* (1990) *Nature* **346**, p183-187).

The APC that is typically present in the method may be from the same individual as the T cell or from a different host. The APC may be a naturally occurring APC or an artificial APC. The APC is a cell that is capable of presenting the peptide to a T cell. It is typically a B cell, dendritic cell or macrophage. It is typically separated from the same sample as the T cell and is

typically co-purified with the T cell. Thus the APC may be present in MCs or PBMCs. The APC is typically a freshly isolated *ex vivo* cell or a cultured cell. It may be in the form of a cell line, such as a short term or immortalised cell line. The APC may express empty MHC class II molecules on its surface.

In the method one or more (different) agents may be used. Typically the T cells derived from the sample can be placed into an assay with all the agents which it is intended to test or the T cells can be divided and placed into separate assays each of which contain one or more of the agents.

The invention also provides the agents such as two or more of any of the agents mentioned herein (e.g. the combinations of agents which are present in the composition agent discussed above) for simultaneous separate or sequential use (eg. for *in vivo* use).

In one embodiment agent *per se* is added directly to an assay comprising T cells and APCs. As discussed above the T cells and APCs in such an assay could be in the form of MCs. When agents that can be recognised by the T cell without the need for presentation by APCs are used then APCs are not required. Analogues which mimic the original (i) or (ii) bound to a MHC molecule are an example of such an agent.

In one embodiment the agent is provided to the APC in the absence of the T cell. The APC is then provided to the T cell, typically after being allowed to present the agent on its surface. The peptide may have been taken up inside the APC and presented, or simply be taken up onto the surface without entering inside the APC.

The duration for which the agent is contacted with the T cells will vary depending on the method used for determining recognition of the peptide. Typically 10^5 to 10^7, preferably $5x10^5$ to 10^6 PBMCs are added to each assay. In the case where agent is added directly to the assay its concentration is from 10^{-1} to 10^3μg/ml, preferably 0.5 to 50μg/ml or 1 to 10μg/ml.

Typically the length of time for which the T cells are incubated with the agent is from 4 to 24 hours, preferably 6 to 16 hours. When using *ex vivo*

PBMCs it has been found that $0.3x10^6$ PBMCs can be incubated in 10μg/ml of peptide for 12 hours at 37°C.

The determination of the recognition of the agent by the T cells may be done by measuring the binding of the agent to the T cells (this can be carried out using any suitable binding assay format discussed herein). Typically T cells which bind the agent can be sorted based on this binding, for example using a FACS machine. The presence of T cells that recognise the agent will be deemed to occur if the frequency of cells sorted using the agent is above a "control" value. The frequency of antigen-experienced T cells is generally 1 in 10^6 to 1 in 10^3, and therefore whether or not the sorted cells are antigen-experienced T cells can be determined.

The determination of the recognition of the agent by the T cells may be measured *in vivo*. Typically the agent is administered to the host and then a response which indicates recognition of the agent may be measured. The agent is typically administered intradermally or epidermally. The agent is typically administered by contacting with the outside of the skin, and may be retained at the site with the aid of a plaster or dressing. Alternatively the agent may be administered by needle, such as by injection, but can also be administered by other methods such as ballistics (e.g. the ballistics techniques which have been used to deliver nucleic acids). EP-A-0693119 describes techniques that can typically be used to administer the agent. Typically from 0.001 to 1000 μg, for example from 0.01 to 100 μg or 0.1 to 10 μg of agent is administered.

In one embodiment a product can be administered which is capable of providing the agent *in vivo*. Thus a polynucleotide capable of expressing the agent can be administered, typically in any of the ways described above for the administration of the agent. The polynucleotide typically has any of the characteristics of the polynucleotide provided by the invention which is discussed below. The agent is expressed from the polynucleotide *in vivo*. Typically from 0.001 to 1000 μg, for example from 0.01 to 100 μg or 0.1 to 10 μg of polynucleotide is administered.

Recognition of the agent administered to the skin is typically indicated by the occurrence of inflammation (e.g. induration, erythema or oedema) at the site of administration. This is generally measured by visual examination of the site.

The method of diagnosis based on the detection of an antibody that binds the agent is typically carried out by contacting a sample from the individual (such as any of the samples mentioned here, optionally processed in any manner mentioned herein) with the agent and determining whether an antibody in the sample binds the agent, such a binding indicating that the individual has, or is susceptible to coeliac disease. Any suitable format of binding assay may be used, such as any such format mentioned herein.

Therapy

The identification of the immunodominant epitope and other epitopes described herein allows therapeutic products to be made which target the T cells which recognise this epitope (such T cells being ones which participate in the immune response against gliadin). These findings also allow the prevention or treatment of coeliac disease by suppressing (by tolerisation) an antibody or T cell response to the epitope(s).

Certain agents of the invention bind the TCR that recognises the epitope of the invention (as measured using any of the binding assays discussed above) and cause tolerisation of the T cell that carries the TCR. Such agents, optionally in association with a carrier, can therefore be used to prevent or treat coeliac disease.

Generally tolerisation can be caused by the same peptides which can (after being recognised by the TCR) cause antigen specific functional activity of the T cell (such as any such activity mentioned herein, e.g. secretion of cytokines). Such agents cause tolerisation when they are presented to the immune system in a 'tolerising' context.

Tolerisation leads to a decrease in the recognition of a T cell or antibody epitope by the immune system. In the case of a T cell epitope this can be caused by the deletion or anergising of T cells that recognise the epitope. Thus T cell

activity (for example as measured in suitable assays mentioned herein) in response to the epitope is decreased. Tolerisation of an antibody response means that a decreased amount of specific antibody to the epitope is produced when the epitope is administered.

Methods of presenting antigens to the immune system in such a context are known and are described for example in Yoshida et al. Clin. Immunol. Immunopathol. 82, 207-215 (1997), Thurau et al. Clin. Exp. Immunol. 109, 370-6 (1997), and Weiner et al. Res. Immunol. 148, 528-33 (1997). In particular certain routes of administration can cause tolerisation, such as oral, nasal or intraperitoneal. Particular products which cause tolerisation may be administered (e.g. in a composition that also comprises the agent) to the individual. Such products include cytokines, such as cytokines that favour a Th2 response (e.g. IL-4, TGF-β or IL-10). Products or agent may be administered at a dose that causes tolerisation.

The invention provides a protein that comprises a sequence able to act as an antagonist of the T cell (which T cell recognises the agent). Such proteins and such antagonists can also be used to prevent or treat coeliac disease. The antagonist will cause a decrease in the T cell response. In one embodiment, the antagonist binds the TCR of the T cell (generally in the form of a complex with HLA-DQ2 or -DQ8) but instead of causing normal functional activation causing an abnormal signal to be passed through the TCR intracellular signalling cascade, which causes the T cell to have decreased function activity (e.g. in response to recognition of an epitope, typically as measured by any suitable assay mentioned herein).

In one embodiment the antagonist competes with epitope to bind a component of MHC processing and presentation pathway, such as an MHC molecule (typically HLA-DQ2 or -DQ8). Thus the antagonist may bind HLA-DQ2 or -DQ8 (and thus be a peptide presented by this MHC molecule), such as peptide TP (Table 10) or a homologue thereof.

Methods of causing antagonism are known in the art. In one embodiment the antagonist is a homologue of the epitopes mentioned above and may have any

of the sequence, binding or other properties of the agent (particularly analogues). The antagonists typically differ from any of the above epitopes (which are capable of causing a normal antigen specific function in the T cell) by 1, 2, 3, 4 or more mutations (each of which may be a substitution, insertion or deletion). Such antagonists are termed "altered peptide ligands" or "APL" in the art. The mutations are typically at the amino acid positions that contact the TCR.

The antagonist may differ from the epitope by a substitution within the sequence that is equivalent to the sequence represented by amino acids 65 to 67 of A-gliadin (such antagonists are shown in Table 9). Thus preferably the antagonist has a substitution at the equivalent of position 64, 65 or 67. Preferably the substitution is 64W, 67W, 67M or 65T.

Since the T cell immune response to the epitope of the invention in an individual is polyclonal, more than one antagonist may need to be administered to cause antagonism of T cells of the response which have different TCRs. Therefore the antagonists may be administered in a composition which comprises at least 2, 4, 6 or more different antagonists, which each antagonise different T cells.

The invention also provides a method of identifying an antagonist of a T cell (which recognises the agent), comprising contacting a candidate substance with the T cell and detecting whether the substance causes a decrease in the ability of the T cell to undergo an antigen specific response (e.g. using any suitable assay mentioned herein), the detecting of any such decrease in said ability indicating that the substance is an antagonist.

In one embodiment, the antagonists (including combinations of antagonists to a particular epitope) or tolerising (T cell and antibody tolerising) agents are present in a composition comprising at least 2, 4, 6 or more antagonists or agents which antagonise or tolerise to different epitopes of the invention, for example to the combinations of epitopes discussed above in relation to the agents which are a product comprising more than one substance.

Testing whether a composition is capable of causing coeliac disease

As mentioned above the invention provides a method of determining whether a composition is capable of causing coeliac disease comprising detecting the presence of a protein sequence which is capable of being modified by a transglutaminase to as sequence comprising the agent or epitope of the invention (such transglutaminase activity may be a human intestinal transglutaminase activity). Typically this is performed by using a binding assay in which a moiety which binds to the sequence in a specific manner is contacted with the composition and the formation of sequence/moiety complex is detected and used to ascertain the presence of the agent. Such a moiety may be any suitable substance (or type of substance) mentioned herein, and is typically a specific antibody. Any suitable format of binding assay can be used (such as those mentioned herein).

In one embodiment, the composition is contacted with at least 2, 5, 10 or more antibodies which are specific for epitopes of the invention from different gliadins, for example a panel of antibodies capable of recognising the combinations of epitopes discussed above in relation to agents of the invention which are a product comprising more than one substance.

The composition typically comprises material from a plant that expresses a gliadin which is capable of causing coeliac disease (for example any of the gliadins or plants mentioned herein). Such material may be a plant part, such as a harvested product (e.g. seed). The material may be processed products of the plant material (e.g. any such product mentioned herein), such as a flour or food that comprises the gliadin. The processing of food material and testing in suitable binding assays is routine, for example as mentioned in Kricka LJ, J. Biolumin. Chemilumin. 13, 189-93 (1998).

Binding assays

The determination of binding between any two substances mentioned herein may be done by measuring a characteristic of either or both substances that changes upon binding, such as a spectroscopic change.

The binding assay format may be a 'band shift' system. This involves determining whether the presence of one substance (such as a candidate substance) advances or retards the progress of the other substance during gel electrophoresis.

The format may be a competitive binding method which determines whether the one substance is able to inhibit the binding of the other substance to an agent which is known to bind the other substance, such as a specific antibody.

Mutant gliadin proteins

The invention provides a gliadin protein in which an epitope sequence of the invention, or sequence which can be modified by a transglutaminase to provide such a sequence has been mutated so that it no longer causes, or is recognised by, a T cell response that recognises the epitope. In this context the term recognition refers to the TCR binding the epitope in such a way that normal (not antagonistic) antigen-specific functional activity of the T cell occurs.

Methods of identifying equivalent epitopes in other gliadins are discussed above. The wild type of the mutated gliadin is one which causes coeliac disease. Such a gliadin may have homology with SEQ ID NO:3, for example to the degree mentioned above (in relation to the analogue) across all of SEQ ID NO:3 or across 15, 30, 60, 100 or 200 contiguous amino acids of SEQ ID NO:3. Likewise, for other non-A-gliadins, homology will be present between the mutant and the native form of that gliadin. The sequences of other natural gliadin proteins are known in the art.

The mutated gliadin will not cause coeliac disease or will cause decreased symptoms of coeliac disease. Typically the mutation decreases the ability of the epitope to induce a T cell response. The mutated epitope may have a decreased binding to HLA-DQ2 or -DQ8, a decreased ability to be presented by an APC or a decreased ability to bind to or to be recognised (i.e. cause antigen-specific functional activity) by T cells that recognise the agent. The mutated gliadin or epitope will therefore show no or reduced recognition in any of the assays mentioned herein in relation to the diagnostic aspects of the invention.

The mutation may be one or more deletions, additions or substitutions of length 1 to 3, 4 to 6, 6 to 10, 11 to 15 or more in the epitope, for example across sequence SEQ ID NO:2 or across any of SEQ ID NOS: 18-22, 31-36, 39-44, and 46; or across equivalents thereof. Preferably the mutant gliadin has at least one mutation in the sequence SEQ ID NO:1. A preferred mutation is at position 65 in A-gliadin (or in an equivalent position in other gliadins). Typically the naturally occurring glutamine at this position is substituted to any of the amino acids shown in Table 3, preferably to histidine, tyrosine, tryptophan, lysine, proline, or arginine.

The invention thus also provides use of a mutation (such any of the mutations in any of the sequences discussed herein) in an epitope of a gliadin protein, which epitope is an epitope of the invention, to decrease the ability of the gliadin protein to cause coeliac disease.

In one embodiment the mutated sequence is able to act as an antagonist. Thus the invention provides a protein that comprises a sequence which is able to bind to a T cell receptor, which T cell receptor recognises an agent of the invention, and which sequence is able to cause antagonism of a T cell that carries such a T cell receptor.

The invention also provides proteins which are fragments of the above mutant gliadin proteins, which are at least 15 amino acids long (e.g. at least 30, 60, 100, 150, 200, or 250 amino acids long) and which comprise the mutations discussed above which decrease the ability of the gliadin to be recognised. Any of the mutant proteins (including fragments) mentioned herein may also be present in the form of fusion proteins, for example with other gliadins or with non-gliadin proteins.

The equivalent wild type protein to the mutated gliadin protein is typically from a graminaceous monocotyledon, such as a plant of genus Triticum, e.g. wheat, rye, barley, oats or triticale. The protein is typically an α, αβ, β, γ or ω gliadin. The gliadin may be an A-gliadin.

Kits

The invention also provides a kit for carrying out the method comprising one or more agents and optionally a means to detect the recognition of the agent by the T cell. Typically the different agents are provided for simultaneous, separate or sequential use. Typically the means to detect recognition allows or aids detection based on the techniques discussed above.

Thus the means may allow detection of a substance secreted by the T cells after recognition. The kit may thus additionally include a specific binding moiety for the substance, such as an antibody. The moiety is typically specific for IFN-γ. The moiety is typically immobilised on a solid support. This means that after binding the moiety the substance will remain in the vicinity of the T cell which secreted it. Thus "spots" of substance/moiety complex are formed on the support, each spot representing a T cell which is secreting the substance. Quantifying the spots, and typically comparing against a control, allows determination of recognition of the agent.

The kit may also comprise a means to detect the substance/moiety complex. A detectable change may occur in the moiety itself after binding the substance, such as a colour change. Alternatively a second moiety directly or indirectly labelled for detection may be allowed to bind the substance/moiety complex to allow the determination of the spots. As discussed above the second moiety may be specific for the substance, but binds a different site on the substance than the first moiety.

The immobilised support may be a plate with wells, such as a microtitre plate. Each assay can therefore be carried out in a separate well in the plate.

The kit may additionally comprise medium for the T cells, detection moieties or washing buffers to be used in the detection steps. The kit may additionally comprise reagents suitable for the separation from the sample, such as the separation of PBMCs or T cells from the sample. The kit may be designed to allow detection of the T cells directly in the sample without requiring any separation of the components of the sample.

The kit may comprise an instrument which allows administration of the agent, such as intradermal or epidermal administration. Typically such an

instrument comprises plaster, dressing or one or more needles. The instrument may allow ballistic delivery of the agent. The agent in the kit may be in the form of a pharmaceutical composition.

The kit may also comprise controls, such as positive or negative controls. The positive control may allow the detection system to be tested. Thus the positive control typically mimics recognition of the agent in any of the above methods. Typically in the kits designed to determine recognition *in vitro* the positive control is a cytokine. In the kit designed to detect *in vivo* recognition of the agent the positive control may be antigen to which most individuals should response.

The kit may also comprise a means to take a sample containing T cells from the host, such as a blood sample. The kit may comprise a means to separate mononuclear cells or T cells from a sample from the host.

Polynucleotides, cells, transgenic mammals and antibodies

The invention also provides a polynucleotide which is capable of expression to provide the agent or mutant gliadin proteins. Typically the polynucleotide is DNA or RNA, and is single or double stranded. The polynucleotide will preferably comprise at least 50 bases or base pairs, for example 50 to 100, 100 to 500, 500 to 1000 or 1000 to 2000 or more bases or base pairs. The polynucleotide therefore comprises a sequence which encodes the sequence of SEQ ID NO: 1 or 2 or any of the other agents mentioned herein. To the 5' and 3' of this coding sequence the polynucleotide of the invention has sequence or codons which are different from the sequence or codons 5' and 3' to these sequences in the corresponding gliadin gene.

5' and/or 3' to the sequence encoding the peptide the polynucleotide has coding or non-coding sequence. Sequence 5' and/or 3' to the coding sequence may comprise sequences which aid expression, such as transcription and/or translation, of the sequence encoding the agent. The polynucleotide may be capable of expressing the agent prokaryotic or eukaryotic cell. In one

embodiment the polynucleotide is capable of expressing the agent in a mammalian cell, such as a human, primate or rodent (e.g. mouse or rat) cell.

A polynucleotide of the invention may hybridise selectively to a polynucleotide that encodes SEQ ID NO:3 at a level significantly above background. Selective hybridisation is typically achieved using conditions of medium to high stringency (for example 0.03M sodium chloride and 0.03M sodium citrate at from about 50°C to about 60°C). However, such hybridisation may be carried out under any suitable conditions known in the art (see Sambrook *et al* (1989), Molecular Cloning: A Laboratory Manual). For example, if high stringency is required, suitable conditions include 0.2 x SSC at 60°C. If lower stringency is required, suitable conditions include 2 x SSC at 60°C.

Agents or proteins of the invention may be encoded by the polynucleotides described herein.

The polynucleotide may form or be incorporated into a replicable vector. Such a vector is able to replicate in a suitable cell. The vector may be an expression vector. In such a vector the polynucleotide of the invention is operably linked to a control sequence which is capable of providing for the expression of the polynucleotide. The vector may contain a selectable marker, such as the ampicillin resistance gene.

The polynucleotide or vector may be present in a cell. Such a cell may have been transformed by the polynucleotide or vector. The cell may express the agent. The cell will be chosen to be compatible with the said vector and may for example be a prokaryotic (bacterial), yeast, insect or mammalian cell. The polynucleotide or vector may be introduced into host cells using conventional techniques including calcium phosphate precipitation, DEAE-dextran transfection, or electroporation.

The invention provides processes for the production of the proteins of the invention by recombinant means. This may comprise (a) cultivating a transformed cell as defined above under conditions that allow the expression of the protein; and preferably (b) recovering the expressed polypeptide. Optionally, the polypeptide may be isolated and/or purified, by techniques known in the art.

The invention also provides TCRs which recognise (or bind) the agent, or fragments thereof which are capable of such recognition (or binding). These can be present in the any form mentioned herein (e.g. purity) discussed herein in relation to the protein of the invention. The invention also provides T cells which express such TCRs which can be present in any form (e.g. purity) discussed herein for the cells of the invention.

The invention also provides monoclonal or polyclonal antibodies which specifically recognise the agents (such as any of the epitopes of the invention) and which recognise the mutant gliadin proteins (and typically which do not recognise the equivalent wild-type gliadins) of the invention, and methods of making such antibodies. Antibodies of the invention bind specifically to these substances of the invention.

For the purposes of this invention, the term "antibody" includes antibody fragments such as Fv, F(ab) and $F(ab)_2$ fragments, as well as single-chain antibodies.

A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the serum. The animal may therefore be inoculated with the immunogen, blood subsequently removed from the animal and the IgG fraction purified. A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells (Kohler and Milstein (1975) *Nature* **256**, 495-497).

An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in vitro* immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat or mouse. If desired, the

immunogen may be administered as a conjugate in which the immunogen is coupled, for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

The polynucleotide, agent, protein or antibody of the invention, may carry a detectable label. Detectable labels which allow detection of the secreted substance by visual inspection, optionally with the aid of an optical magnifying means, are preferred. Such a system is typically based on an enzyme label which causes colour change in a substrate, for example alkaline phosphatase causing a colour change in a substrate. Such substrates are commercially available, e.g. from BioRad. Other suitable labels include other enzymes such as peroxidase, or protein labels, such as biotin; or radioisotopes, such as ^{32}P or ^{35}S. The above labels may be detected using known techniques.

Polynucleotides, agents, proteins, antibodies or cells of the invention may be in substantially purified form. They may be in substantially isolated form, in which case they will generally comprise at least 80% e.g. at least 90, 95, 97 or 99% of the polynucleotide, peptide, antibody, cells or dry mass in the preparation. The polynucleotide, agent, protein or antibody is typically substantially free of other cellular components. The polynucleotide, agent, protein or antibody may be used in such a substantially isolated, purified or free form in the method or be present in such forms in the kit.

The invention also provides a transgenic non-human mammal which expresses a TCR of the invention. This may be any of the mammals discussed herein (e.g. in relation to the production of the antibody). Preferably the mammal has, or is susceptible, to coeliac disease. The mammal may also express HLA-DQ2 or -DQ8 or HLA-DR3-DQ2 and/or may be given a diet comprising a gliadin which cause coeliac disease (e.g. any of the gliadin proteins mentioned herein). Thus the mammal may act as an animal model for coeliac disease.

The invention also provides a method of identifying a product which is therapeutic for coeliac disease comprising administering a candidate substance to a mammal of the invention which has, or which is susceptible to, coeliac disease

and determining whether substance prevents or treats coeliac disease in the mammal, the prevention or treatment of coeliac disease indicating that the substance is a therapeutic product. Such a product may be used to treat or prevent coeliac disease.

The invention provides therapeutic (including prophylactic) agents or diagnostic substances (the agents, proteins and polynucleotides of the invention). These substances are formulated for clinical administration by mixing them with a pharmaceutically acceptable carrier or diluent. For example they can be formulated for topical, parenteral, intravenous, intramuscular, subcutaneous, intraocular, intradermal, epidermal or transdermal administration. The substances may be mixed with any vehicle which is pharmaceutically acceptable and appropriate for the desired route of administration. The pharmaceutically carrier or diluent for injection may be, for example, a sterile or isotonic solution such as Water for Injection or physiological saline, or a carrier particle for ballistic delivery.

The dose of the substances may be adjusted according to various parameters, especially according to the agent used; the age, weight and condition of the patient to be treated; the mode of administration used; the severity of the condition to be treated; and the required clinical regimen. As a guide, the amount of substance administered by injection is suitably from 0.01 mg/kg to 30 mg/kg, preferably from 0.1 mg/kg to 10 mg/kg.

The routes of administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage for any particular patient and condition.

The substances of the invention may thus be used in a method of treatment of the human or animal body, or in a diagnostic method practised on the human body. In particular they may be used in a method of treating or preventing coeliac disease. The invention also provide the agents for use in a method of manufacture of a medicament for treating or preventing coeliac disease. Thus the invention provides a method of preventing or treating coeliac disease comprising

administering to a human in need thereof a substance of the invention (typically a non-toxic effective amount thereof).

The agent of the invention can be made using standard synthetic chemistry techniques, such as by use of an automated synthesizer. The agent may be made from a longer polypeptide e.g. a fusion protein, which polypeptide typically comprises the sequence of the peptide. The peptide may be derived from the polypeptide by for example hydrolysing the polypeptide, such as using a protease; or by physically breaking the polypeptide. The polynucleotide of the invention can be made using standard techniques, such as by using a synthesiser.

Plant cells and plants that express mutant gliadin proteins or express proteins comprising sequences which can act as antagonists

The cell of the invention may be a plant cell, such as a cell of a graminaceous monocotyledonous species. The species may be one whose wild-type form expresses gliadins, such as any of the gliadin proteins mentioned herein (including gliadins with any degree of homology to SEQ ID NO:3 mentioned herein). Such a gliadin may cause coeliac disease in humans. The cell may be of wheat, maize, oats, rye, rice, barley, triticale, sorghum, or sugar cane. Typically the cell is of the Triticum genus, such as aestivum, spelta, polonicum or monococcum.

The plant cell of the invention is typically one which does not express a wild-type gliadin (such as any of the gliadins mentioned herein which may cause coeliac disease), or one which does not express a gliadin comprising a sequence that can be recognised by a T cell that recognises the agent. Thus if the wild-type plant cell did express such a gliadin then it may be engineered to prevent or reduce the expression of such a gliadin or to change the amino acid sequence of the gliadin so that it no longer causes coeliac disease (typically by no longer expressing the epitope of the invention).

This can be done for example by introducing mutations into 1, 2, 3 or more or all of such gliadin genes in the cell, for example into coding or non-coding (e.g. promoter regions). Such mutations can be any of the type or length

of mutations discussed herein (e.g., in relation to homologous proteins). The mutations can be introduced in a directed manner (e.g., using site directed mutagenesis or homologous recombination techniques) or in a random manner (e.g. using a mutagen, and then typically selecting for mutagenised cells which no longer express the gliadin (or a gliadin sequence which causes coeliac disease)).

In the case of plants or plant cells that express a protein that comprises a sequence able to act as an antagonist such a plant or plant cell may express a wild-type gliadin protein (e.g. one which causes coeliac disease). Preferably though the presence of the antagonist sequence will cause reduced coeliac disease symptoms (such as no symptoms) in an individual who ingests a food comprising protein from the plant or plant cell.

The polynucleotide which is present in (or which was transformed into) the plant cell will generally comprise promoter capable of expressing the mutant gliadin protein the plant cell. Depending on the pattern of expression desired, the promoter may be constitutive, tissue- or stage-specific; and/or inducible. For example, strong constitutive expression in plants can be obtained with the CAMV 35S, Rubisco ssu, or histone promoters. Also, tissue-specific or stage-specific promoters may be used to target expression of protein of the invention to particular tissues in a transgenic plant or to particular stages in its development. Thus, for example seed-specific, root-specific, leaf-specific, flower-specific etc promoters may be used. Seed-specific promoters include those described by Dalta *et al* (Biotechnology Ann. Rev. (1997), 3, pp.269-296). Particular examples of seed-specific promoters are napin promoters (EP-A-0 255, 378), phaseolin promoters, glutenine promoters, helianthenine promoters (WO92/17580), albumin promoters (WO98/45460), oleosin promoters (WO98/45461) and ATS1 and ATS3 promoters (PCT/US98/06798).

The cell may be in any form. For example, it may be an isolated cell, e.g. a protoplast, or it may be part of a plant tissue, e.g. a callus, or a tissue excised from a plant, or it may be part of a whole plant. The cell may be of any type (e.g. of any type of plant part). For example, an undifferentiated cell, such as a callus cell; or a differentiated cell, such as a cell of a type found in embryos, pollen,

roots, shoots or leaves. Plant parts include roots; shoots; leaves; and parts involved in reproduction, such as pollen, ova, stamens, anthers, petals, sepals and other flower parts.

The invention provides a method of obtaining a transgenic plant cell comprising transforming a plant cell with a polynucleotide or vector of the invention to give a transgenic plant cell. Any suitable transformation method may be used (in the case of wheat the techniques disclosed in Vasil V et al, Biotechnology 10, 667-674 (1992) may be used). Preferred transformation techniques include electroporation of plant protoplasts and particle bombardment. Transformation may thus give rise to a chimeric tissue or plant in which some cells are transgenic and some are not.

The cell of the invention or thus obtained cell may be regenerated into a transgenic plant by techniques known in the art. These may involve the use of plant growth substances such as auxins, giberellins and/or cytokinins to stimulate the growth and/or division of the transgenic cell. Similarly, techniques such as somatic embryogenesis and meristem culture may be used. Regeneration techniques are well known in the art and examples can be found in, e.g. US 4,459,355, US 4,536,475, US 5,464,763, US 5, 177,010, US 5, 187,073, EP 267,159, EP 604, 662, EP 672, 752, US 4,945,050, US 5,036,006, US 5,100,792, US 5,371,014, US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956, US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520, US 5,510,318, US 5,204,253, US 5,405,765, EP 442,174, EP 486,233, EP 486,234, EP 539,563, EP 674,725, WO91/02071 and WO 95/06128.

In many such techniques, one step is the formation of a callus, i.e. a plant tissue comprising expanding and/or dividing cells. Such calli are a further aspect of the invention as are other types of plant cell cultures and plant parts. Thus, for example, the invention provides transgenic plant tissues and parts, including embryos, meristems, seeds, shoots, roots, stems, leaves and flower parts. These may be chimeric in the sense that some of their cells are cells of the invention and some are not. Transgenic plant parts and tissues, plants and seeds of the invention may be of any of the plant species mentioned herein.

Regeneration procedures will typically involve the selection of transformed cells by means of marker genes.

The regeneration step gives rise to a first generation transgenic plant. The invention also provides methods of obtaining transgenic plants of further generations from this first generation plant. These are known as progeny transgenic plants. Progeny plants of second, third, fourth, fifth, sixth and further generations may be obtained from the first generation transgenic plant by any means known in the art.

Thus, the invention provides a method of obtaining a transgenic progeny plant comprising obtaining a second-generation transgenic progeny plant from a first-generation transgenic plant of the invention, and optionally obtaining transgenic plants of one or more further generations from the second-generation progeny plant thus obtained.

Progeny plants may be produced from their predecessors of earlier generations by any known technique. In particular, progeny plants may be produced by:

obtaining a transgenic seed from a transgenic plant of the invention belonging to a previous generation, then obtaining a transgenic progeny plant of the invention belonging to a new generation by growing up the transgenic seed; and/or

propagating clonally a transgenic plant of the invention belonging to a previous generation to give a transgenic progeny plant of the invention belonging to a new generation; and/or

crossing a first-generation transgenic plant of the invention belonging to a previous generation with another compatible plant to give a transgenic progeny plant of the invention belonging to a new generation; and optionally

obtaining transgenic progeny plants of one or more further generations from the progeny plant thus obtained.

These techniques may be used in any combination. For example, clonal propagation and sexual propagation may be used at different points in a process that gives rise to a transgenic plant suitable for cultivation. In particular,

repetitive back-crossing with a plant taxon with agronomically desirable characteristics may be undertaken. Further steps of removing cells from a plant and regenerating new plants therefrom may also be carried out.

Also, further desirable characteristics may be introduced by transforming the cells, plant tissues, plants or seeds, at any suitable stage in the above process, to introduce desirable coding sequences other than the polynucleotides of the invention. This may be carried out by the techniques described herein for the introduction of polynucleotides of the invention.

For example, further transgenes may be selected from those coding for other herbicide resistance traits, e.g. tolerance to: Glyphosate (e.g. using an EPSP synthase gene (e.g. EP-A-0 293,358) or a glyphosate oxidoreductase (WO 92/000377) gene); or tolerance to fosametin; a dihalobenzonitrile; glufosinate, e.g. using a phosphinothrycin acetyl transferase (PAT) or glutamine synthase gene (cf. EP-A-0 242,236); asulam, e.g. using a dihydropteroate synthase gene (EP-A-0 369,367); or a sulphonylurea, e.g. using an ALS gene); diphenyl ethers such as acifluorfen or oxyfluorfen, e.g. using a protoporphyrogen oxidase gene); an oxadiazole such as oxadiazon; a cyclic imide such as chlorophthalim; a phenyl pyrazole such as TNP, or a phenopylate or carbamate analogue thereof.

Similarly, genes for beneficial properties other than herbicide tolerance may be introduced. For example, genes for insect resistance may be introduced, notably genes encoding *Bacillus thuringiensis* (*Bt*) toxins. Likewise, genes for disease resistance may be introduced, e.g. as in WO91/02701 or WO95/06128.

Typically, a protein of the invention is expressed in a plant of the invention. Depending on the promoter used, this expression may be constitutive or inducible. Similarly, it may be tissue- or stage-specific, i.e. directed towards a particular plant tissue (such as any of the tissues mentioned herein) or stage in plant development.

The invention also provides methods of obtaining crop products by harvesting, and optionally processing further, transgenic plants of the invention. By crop product is meant any useful product obtainable from a crop plant.

Products that contain mutant gliadin proteins or proteins that comprise sequence capable of acting as an antagonist

The invention provides a product that comprises the mutant gliadin proteins or protein that comprises sequence capable of acting as an antagonist. This is typically derived from or comprise plant parts from plants mentioned herein which express such proteins. Such a product may be obtainable directly by harvesting or indirectly, by harvesting and further processing the plant of the invention. Directly obtainable products include grains. Alternatively, such a product may be obtainable indirectly, by harvesting and further processing. Examples of products obtainable by further processing are flour or distilled alcoholic beverages; food products made from directly obtained or further processed material, e.g. baked products (e.g. bread) made from flour. Typically such food products, which are ingestible and digestible (i.e. non-toxic and of nutrient value) by human individuals.

In the case of food products that comprise the protein which comprises an antagonist sequence the food product may also comprise wild-type gliadin, but preferably the antagonist is able to cause a reduction (e.g. completely) in the coeliac disease symptoms after such food is ingested.

The invention is illustrated by the following nonlimiting Examples:

Example 1

We carried out epitope mapping in Coeliac disease by using a set of 51 synthetic 15-mer peptides that span the complete sequence of a fully characterized a-gliadin, "A-gliadin" (see Table 1). A-Gliadin peptides were also individually treated with tTG to generate products that might mimic those produced in vivo[3]. We also sought to study Coeliac disease patients at the point of initiation of disease relapse to avoid the possibility that epitope "spreading" or "exhaustion" may have occurred, as described in experimental infectious and autoimmune diseases.

Clinical and A-gliadin specific T cell responses with 3 and 10 day bread

challenge

In a pilot study, two subjects with Coeliac disease in remission, defined by absence of serum anti-endomysial antibody (EMA), on a gluten free diet were fed four slices of standard gluten-containing white bread daily in addition to their usual gluten free diet. Subject 1 ceased bread because of abdominal pain, mouth ulcers and mild diarrhoea after three days, but Subject 2 continued for 10 days with only mild nausea at one week. The EMA became positive in Subject 2 one week after the bread challenge, indicating the bread used had caused a relapse of Coeliac disease. But in Subject 1, EMA remained negative up to two months after bread challenge. In both subjects, symptoms that appeared with bread challenge resolved within two days after returning to gluten free diet.

PBMC responses in IFNγ ELISPOT assays to A-gliadin peptides were not found before or during bread challenge. But from the day after bread withdrawal (Day 4) in Subject 1 a single pool of 5 overlapping peptides spanning A-gliadin 51-85 (Pool 3) treated with tTG showed potent IFNγ responses (see Figure 1a). In Subject 1, the PBMC IFNγ response to A-gliadin peptide remained targeted to Pool 3 alone and was maximal on Day 8. The dynamics and magnitude of the response to Pool 3 was similar to that elicited by α-chymotrypsin digested gliadin. PBMC IFNγ responses to tTG-treated Pool 3 were consistently 5 to 12-fold greater than Pool 3 not treated with tTG, and responses to α-chymotrypsin digested gliadin were 3 to 10-fold greater if treated with tTG. In Subject 2, Pool 3 treated with tTG was also the only immunogenic set of A-gliadin peptides on Day 8, but this response was weaker than Subject 1, was not seen on Day 4 and by Day 11 the response to Pool 3 had diminished and other tTG-treated pools of A-gliadin peptides elicited stronger IFNα responses (see Figure 1b).

The pilot study indicated that the initial T cell response in these Coeliac disease subjects was against a single tTG-treated A-gliadin pool of five peptides and was readily measured in peripheral blood. But if antigen exposure is continued for ten days instead of three, T cell responses to other A-gliadin peptides appear, consistent with epitope spreading.

Coeliac disease-specific IFN-g induction by tTG-treated A-gliadin peptides

In five out of six further Coeliac disease subjects on gluten free diet (see Table 1), bread challenge for three days identified tTG-treated peptides in Pool 3, and in particular, peptides corresponding to 56-70 (12) and 60-75 (13) as the sole A-gliadin components eliciting IFNγ from PBMC (see Figure 2). IL-10 ELISPOT assays run in parallel to IFNγ ELISPOT showed no IL-10 response to tTG-treated peptides 12 or 13. In one subject, there were no IFNγ responses to any A-gliadin peptide or α-chymotrypsin digested gliadin before, during or up to four days after bread challenge. In none of these Coeliac disease subjects did EMA status change from baseline when measured for up to two months after bread challenge.

PBMC from four healthy, EMA-negative subjects with the HLA-DQ alleles α1*0501, β1*0201 (ages 28-52, 2 females) who had been challenged for three days with bread after following a gluten free diet for one month, showed no IFNγ responses above the negative control to any of the A-gliadin peptides with or without tTG treatment. Thus, induction of IFNγ in PBMC to tTG-treated Pool 3 and A-gliadin peptides 56-70 (12) and 60-75 (13) were Coeliac disease specific (7/8 vs. 0/4, $p<0.01$ by Chi-squared analysis).

Fine mapping of the minimal A-gliadin T cell epitope

tTG-treated peptides representing truncations of A-gliadin 56-75 revealed that the same core peptide sequence QPQLP (SEQ ID NO:9) was essential for antigenicity in all of the five Coeliac disease subjects assessed (see Figure 3). PBMC IFNγ responses to tTG-treated peptides spanning this core sequence beginning with the 7-mer PQPQLPY (SEQ ID NO:4) and increasing in length, indicated that the tTG-treated 17-mer QLQPFPQPQLPYPQPQS (SEQ ID NO:10) (A-gliadin 57-73) possessed optimal activity in the IFNγ ELISPOT (see Figure 4).

Deamidation of Q65 by tTG generates the immunodominant T cell epitope in A-gliadin

HPLC analysis demonstrated that tTG treatment of A-gliadin 56-75 generated a single product that eluted marginally later than the parent peptide. Amino acid sequencing indicated that out of the six glutamine (Q) residues contained in A-gliadin 56-75, Q65 was preferentially deamidated by tTG (see Figure 5). Bioactivity of peptides corresponding to serial expansions from the core A-gliadin 62-68 sequence in which glutamate (E) replaced Q65, was equivalent to the same peptides with Q65 after tTG-treatment (see Figure 4a). Replacement of Q57 and Q72 by E together or alone, with E65 did not enhance antigenicity of the 17-mer in the three Coeliac disease subjects studied (see Figure 6). Q57 and Q72 were investigated because glutamine residues followed by proline in gliadin peptides are not deamidated by tTG in vitro (W. Vader et al, Proceedings 8th International Symposium Coeliac Disease). Therefore, the immunodominant T cell epitope was defined as QLQPFPQPELPYPQPQS (SEQ ID NO:2).

Immunodominant T cell epitope response is DQ2-restricted and CD4 dependent

In two Coeliac disease subjects homozygous for HLA-DQ α1*0501, β1*0201, anti-DQ monoclonal antibody blocked the ELISPOT IFNγ response to tTG-treated A-gliadin 56-75, but anti-DP and -DR antibody did not (see Figure 7). Anti-CD4 and anti-CD8 magnetic bead depletion of PBMC from two Coeliac disease subjects indicated the IFNγ response to tTG-treated A-gliadin 56-75 is CD4 T cell-mediated.

Discussion

In this study we describe a rather simple dietary antigen challenge using standard white bread to elicit a transient population of CD4 T cells in peripheral blood of Coeliac disease subjects responsive to a tTG-treated A-gliadin 17-mer with the sequence: QLQPFPQPELPYPQPQS (SEQ ID NO:2) (residues 57-73). The immune response to A-gliadin 56-75 (Q→E65) is restricted to the Coeliac disease-associated HLA allele, DQ α1*0501, β1*0201. Tissue transglutaminase action in vitro selectively deamidates Q65. Elicited peripheral blood IFNg

responses to synthetic A-gliadin peptides with the substitution Q→E65 is equivalent to tTG-treated Q65 A-gliadin peptides; both stimulate up to 10-fold more T cells in the IFNg ELISPOT than unmodified Q65 A-gliadin peptides.

We have deliberately defined this Coeliac disease-specific T cell epitope using in vivo antigen challenge and short-term ex vivo immune assays to avoid the possibility of methodological artifacts that may occur with the use of T cell clones in epitope mapping. Our findings indicate that peripheral blood T cell responses to ingestion of gluten are rapid but short-lived and can be utilized for epitope mapping. In vivo antigen challenge has also shown there is a temporal hierarchy of immune responses to A-gliadin peptides; A-gliadin 57-73 modified by tTG not only elicits the strongest IFNg response in PBMC but it is also the first IFNg response to appear.

Because we have assessed only peptides spanning A-gliadin, there may be other epitopes in other gliadins of equal or greater importance in the pathogenesis of Coeliac disease. Indeed, the peptide sequence at the core of the epitope in A-gliadin that we have identified PQPQLPY (SEQ ID NO:4) is shared by several other gliadins (SwissProt and Trembl accession numbers: P02863, Q41528, Q41531, Q41533, Q9ZP09, P04722, P04724, P18573). However, A-gliadin peptides that have previously been shown to possess bioactivity in biopsy challenge and in vivo studies (for example: 31-43, 44-55, and 206-217)[4,5] did not elicit IFNg responses in PBMC following three day bread challenge in Coeliac disease subjects. These peptides may be "secondary" T cell epitopes that arise with spreading of the immune response.

Example 2

The effect on T cell recognition of substitutions in the immunodominant epitope

The effect of substituting the glutamate at position 65 in the 57-73 A-gliadin epitope was determined by measuring peripheral blood responses against the substituted epitopes in an IFNγ ELISPOT assay using synthetic peptides (at 50 μg/ml). The responses were measured in 3 Coeliac disease subjects 6 days after commencing gluten challenge (4 slices bread daily for 3 days). Results are

shown in table 3 and Figure 8. As can be seen substitution of the glutamate to histidine, tyrosine, tryptophan, lysine, proline or arginine stimulated a response whose magnitude was less than 10% of the magnitude of the response to the immunodominant epitope. Thus mutation of A-gliadin at this position could be used to produce a mutant gliadin with reduce or absent immunoreactivity.

Example 3

Testing the immunoreactivity of equivalent peptides from other naturally occurring gliadins

The immunoreactivity of equivalent peptides form other naturally occurring wheat gliadins was assessed using synthetic peptides corresponding to the naturally occurring sequences which were then treated with transglutaminase. These peptides were tested in an ELISPOT in the same manner and with PBMCs from the same subjects as described in Example 2. At least five of the peptides show immunoreactivity comparable to the A-gliadin 57-73 E65 peptide (after transglutaminase treatment) indicating that other gliadin proteins in wheat are also likely to induce this Coeliac disease-specific immune response (Table 4 and Figure 9).

Methods

Subjects: Patients used in the study attended a Coeliac Clinic in Oxford, United Kingdom. Coeliac disease was diagnosed on the basis of typical small intestinal histology, and normalization of symptoms and small intestinal histology with gluten free diet.

Tissue typing: Tissue typing was performed using DNA extracted from EDTA-anticoagulated peripheral blood. HLA-DQA and DQB genotyping was performed by PCR using sequence-specific primer mixes[6-8].

Anti-endomysial antibody assay: EMA were detected by indirect immunofluorescence using patient serum diluted 1:5 with monkey oesophagus,

followed by FITC-conjugated goat anti-human IgA. IgA was quantitated prior to EMA, none of the subjects were IgA deficient.

Antigen Challenge: Coeliac disease subjects following a gluten free diet, consumed 4 slices of gluten-containing bread (50g/slice, Sainsbury's "standard white sandwich bread") daily for 3 or 10 days. EMA was assessed the week before and up to two months after commencing the bread challenge. Healthy subjects who had followed a gluten free diet for four weeks, consumed their usual diet including four slices of gluten-containing bread for three days, then returned to gluten free diet for a further six days.

IFNγ and IL-10 ELISPOT: PBMC were prepared from 50-100 ml of venous blood by Ficoll-Hypaque density centrifugation. After three washes, PBMC were resuspended in complete RPMI containing 10% heat inactivated human AB serum. ELISPOT assays for single cell secretion of IFNγ and IL-10 were performed using commercial kits (Mabtech; Stockholm, Sweden) with 96-well plates (MAIP-S-45; Millipore, Bedford, MA) according to the manufacturers instructions (as described elsewhere[9]) with $2\text{-}5x10^5$ (IFNγ) or $0.4\text{-}1x10^5$ (IL-10) PBMC in each well. Peptides were assessed in duplicate wells, and Mycobacterium tuberculosis purified protein derivative (PPD RT49) (Serum Institute; Copenhagen, Denmark) (20 μg/ml) was included as a positive control in all assays.

Peptides: Synthetic peptides were purchased from Research Genetics (Huntsville, Alabama) Mass-spectroscopy and HPLC verified peptides' authenticity and >70% purity. Digestion of gliadin (Sigma; G-3375) (100 mg/ml) with α-chymotrypsin (Sigma; C-3142) 200:1 (w/w) was performed at room temperature in 0.1 M NH_4HCO_3 with 2M urea and was halted after 24 h by heating to 98°C for 10 minutes. After centrifugation (13,000g, 10 minutes), the gliadin digest supernatant was filter-sterilized (0.2 mm). Digestion of gliadin was verified by SDS-PAGE and protein concentration assessed. α-Chymotrypsin-digested

gliadin (640 μg/ml) and synthetic gliadin peptides (15-mers: 160 μg/ml, other peptides: 0.1 mM) were individually treated with tTG (Sigma; T-5398) (50 μg/ml) in PBS + $CaCl_2$ 1 mM for 2 h at 37°C. Peptides and peptide pools were aliquotted into sterile 96-well plates and stored frozen at -20°C until use.

Amino acid sequencing of peptides: Reverse phase HPLC was used to purify the peptide resulting from tTG treatment of A-gliadin 56-75. A single product was identified and subjected to amino acid sequencing (automated sequencer Model 494A, Applied Biosystems, Foster City, California). The sequence of unmodified G56-75 was confirmed as: LQLQPFPQPQLPYPQPQSFP (SEQ ID NO:5), and tTG treated G56-75 was identified as: LQLQPFPQPELPYPQPQSFP (SEQ ID NO:11). Deamidation of glutamyl residues was defined as the amount (pmol) of glutamate recovered expressed as a percent of the combined amount of glutamine and glutamate recovered in cycles 2, 4, 8, 10, 15 and 17 of the amino acid sequencing. Deamidation attributable to tTG was defined as (% deamidation of glutamine in the tTG treated peptide - % deamidation in the untreated peptide) / (100 - % deamidation in the untreated peptide).

CD4/CD8 and HLA Class II Restriction: Anti-CD4 or anti-CD8 coated magnetic beads (Dynal, Oslo, Norway) were washed four times with RPMI then incubated with PBMC in complete RPMI containing 10% heat inactivated human AB serum ($5x10^6$ cells/ml) for 30 minutes on ice. Beads were removed using a magnet and cells remaining counted. In vivo HLA-class II restriction of the immune response to tTG-treated A-gliadin 56-75 was established by incubating PBMC ($5x10^6$ cells/ml) with anti-HLA-DR (L243), -DQ (L2), and -DP (B7.21) monoclonal antibodies (10 μg/ml) at room temperature for one hour prior to the addition of peptide.

Example 4

Mucosal integrin expression by gliadin -specific peripheral blood lymphocytes

Interaction between endothelial and lymphocyte adressins facilitates homing

of organ-specific lymphocytes. Many adressins are known. The heterodimer $\alpha_4\beta_7$ is specific for lamina propria gut and other mucosal lymphocytes, and $\alpha^E\beta_7$ is specific and intra-epithelial lymphocytes in the gut and skin. Approximately 30% of peripheral blood CD4 T cells express $\alpha_4\beta_7$ and are presumed to be in transit to a mucosal site, while 5% of peripheral blood T cells express $\alpha^E\beta_7$. Immunomagnetic beads coated with antibody specific for α^E or β_7 deplete PBMC of cells expressing $\alpha^E\beta_7$ or $\alpha^E\beta_7$ and $\alpha_4\beta_7$, respectively. In combination with ELISpot assay, immunomagnetic bead depletion allows determination of gliadin-specific T cell addressin expression that may identify these cells as homing to a mucosal surface. Interestingly, gluten challenge in vivo is associated with rapid influx of CD4 T cells to the small intestinal lamina propria (not intra-epithelial sites), where over 90% lymphocytes express $\alpha_4\beta_7$.

Immunomagnetic beads were prepared and used to deplete PBMC from coeliac subjects on day 6 or 7 after commencing 3 day gluten challenge. FACS analysis demonstrated α^E beads depleted approximately 50% of positive CD4 T cells, while β_7 beads depleted all β_7 positive CD4 T cells. Depletion of PBMC using CD4- or β_7-beads, but not CD8- or α^E-beads, abolished responses in the interferon gamma ELISpot. tTG gliadin and PPD responses were abolished by CD4 depletion, but consistently affected by integrin-specific bead depletion.

Thus A-gliadin 57-73 QE65-specific T cells induced after gluten challenge in coeliac disease express the integrin, $\alpha_4\beta_7$, present on lamina propria CD4 T cells in the small intestine.

Example 5

Optimal T cell Epitope Length

Previous data testing peptides from 7 to 17 amino acids in length spanning the

core of the dominant T cell epitope in A-gliadin indicated that the 17mer, A-gliadin 57-73 QE65 (SEQ ID NO:2) induced maximal responses in the interferon gamma Elispot using peripheral blood mononuclear cells (PBMC) from coeliac volunteers 6 days after commencing a 3-day gluten challenge.

Peptides representing expansions form the core sequence of the dominant T cell epitope in A-gliadin were assessed in the IFN gamma ELISPOT using peripheral blood mononuclear cells (PBMC) from coeliac volunteers in 6 days after commencing a 3-day gluten challenge (n=4). Peptide 13: A-gliadin 59-71 QE65 (13mer), peptide 15: 58-72 QE65 (15mer), ..., peptide 27: 52-78 SE65 (27mer).

As shown in Figure 11 expansion of the A-gliadin 57-73 QE65 sequence does not substantially enhance response in the IFNgamma Elispot. Subsequent Examples characterise the agonist and antagonist activity of A-gliadin 57-73 QE65 using 17mer peptides.

Example 6

Comparison of A-gliadin 57-73 QE65 with other DQ2-restricted T cell epitopes in coeliac disease

Dose response studies were performed using peptides corresponding to unmodified and transglutaminase-treated peptides corresponding to T cell epitopes of gluten-specific T cell clones and lines from intestinal biopsies of coeliac subjects. Responses to peptides were expressed as percent of response to A-gliadin 57-73 QE65. All subjects were HLA-DQ2+ (none were DQ8+).

The studies indicate that A-gliadin 57-73 QE65 is the most potent gliadin peptide for induction of interferon gamma in the ELISpot assay using coeliac PBMC after gluten challenge (see Figure 12a-h, and Tables 5 and 6). The second and third epitopes are suboptimal fragments of larger peptides i.e. A-gliadin 57-73 QE65 and GDA4_WHEAT P04724-84-100 QE92. The epitope is only modestly bioactive (approximately $1/20^{th}$ as active as A-gliadin 57-73 QE65 after blank is subtracted).

A-gliadin 57-73 QE65 is more potent than other known T cell epitopes in coeliac disease. There are 16 polymorphisms of A-gliadin 57-73 (including the sequence PQLPY (SEQ ID NO:12)) amongst sequenced gliadin genes, their bioactivity is assessed next.

Example 7

Comparison of gliadin- and A-gliadin 57-73 QE65-specific responses in peripheral blood

The relative contribution of the dominant epitope, A-gliadin 57-73 QE65, to the total T cell response to gliadin in coeliac disease is a critical issue. Pepsin-trypsin and chymotrypsin-digested gliadin have been traditionally used as antigen for development of T cell lines and clones in coeliac disease. However, it is possible that these proteases may cleave through certain peptide epitopes. Indeed, chymotrypsin digestion of recombinant α9-gliadin generates the peptide QLQPFPQPELPY (SEQ ID NO:13), that is a truncation of the optimal epitope sequence QLQPFPQPELPYPQPQS (SEQ ID NO:2) (see above). Transglutaminase-treatment substantially increases the potency of chymotrypsin-digested gliadin in proliferation assays of gliadin-specific T cell clones and lines. Hence, transglutaminase-treated chymotrypsin-digested gliadin (tTG gliadin) may not be an ideal antigen, but responses against this mixture may approximate the "total" number of peripheral blood lymphocyte specific for gliadin. Comparison of responses against A-gliadin 57-73 QE65 and tTG gliadin in the ELISpot assay gives an indication of the contribution of this dominant epitope to the overall immune response to gliadin in coeliac disease, and also be a measure of epitope spreading.

PBMC collected on day 6 or 7 after commencing gluten challenge in 4 coeliac subjects were assessed in dose response studies using chymotrypsin-digested gliadin +/- tTG treatment and compared with ELISpot responses to an optimal concentration of A-gliadin 57-73 QE65 (25mcg/ml). TTG treatment of gliadin enhanced PBMC responses in the ELISpot approximately 10-fold (tTG was comparable to blank when assessed alone) (see Figure 13a-c). In the four coeliac subjects studied, A-gliadin 57-73 QE65 (25 mcg/ml) elicited responses between 14 and 115% those of tTG gliadin (500 mcg/ml), and the greater the response to A-gliadin 57-73 QE65 the greater proportion it represented of the tTG gliadin response.

Relatively limited data suggest that A-gliadin 57-73 QE65 responses are comparable to tTG gliadin in some subjects. Epitope spreading associated with more evolved anti-gliadin T cell responses may account for the smaller contribution of A-gliadin 57-73 QE65 to "total" gliadin responses in peripheral blood in some individuals. Epitope spreading may be maintained in individuals with less strictly gluten free diets.

Example 8

Definition of gliadin peptides bioactive in coeliac disease: polymorphisms of A-gliadin 57-73

Overlapping 15mer peptides spanning the complete sequence of A-gliadin were assessed in order to identify the immunodominant sequence in coeliac disease. A-gliadin was the first fully sequenced alpha gliadin protein and gene, but is one of approximately 30-50 related alpha gliadin proteins in wheat. Twenty five distinct alpha-gliadin genes have been identified by searching protein data bases, Swiss-Prot and TREMBL describing a further 8 alpha-gliadins. Contained within these 25 alpha-gliadins, there are 16 distinct polymorphisms of the sequence corresponding to A-gliadin 57-73 (see Table 7).

Synthetic peptides corresponding to these 16 polymorphisms, in an unmodified form, after treatment with transglutaminase in vitro, as well as with glutamate substituted at position 10 (equivalent to QE65 in A-gliadin 57-73) were assessed using PBMC from coeliac subjects, normally following a gluten free diet, day 6 or 7 after gluten challenge in interferon gamma ELISpot assays. Glutamate-substituted peptides were compared at three concentrations (2.5, 25 and 250 mcg/ml), unmodified peptide and transglutaminase-treated peptides were assessed at 25 mcg/ml only. Bioactivity was expressed as % of response associated with A-gliadin 57-73 QE65 25 mcg/ml in individual subjects (n=4). (See Fig 14).

Bioactivity of "wild-type" peptides was substantially increased (>5-fold) by treatment with transglutaminase. Transglutaminase treatment of wild-type peptides resulted in bioactivity similar to that of the same peptides substituted

with glutamate at position 10. Bioactivities of five glutamate-substituted peptides (B, C, K, L, M), were >70% that of A-gliadin 57-73 QE65 (A), but none was significantly more bioactive than A-gliadin 57-73 QE65. PBMC responses to glutamate-substituted peptides at concentrations of 2.5 and 250 mcg/ml were comparable to those at 25 mcg/ml. Six glutamate-substituted gliadin peptides (H, I, J, N, O, P) were <15% as bioactive as A-gliadin 57-73 QE65. Other peptides were intermediate in bioactivity.

At least six gliadin-derived peptides are equivalent in potency to A-gliadin 57-73 QE65 after modification by transglutaminase. Relatively non-bioactive polymorphisms of A-gliadin 57-73 also exist. These data indicate that transglutaminase modification of peptides from several gliadins of *Triticum aestivum*, *T. uartu* and *T. spelta* may be capable of generating the immunodominant T cell epitope in coeliac disease.

Genetic modification of wheat to generate non-coeliac-toxic wheat may likely require removal or modification of multiple gliadin genes. Generation of wheat containing gliadins or other proteins or peptides incorporating sequences defining altered peptide ligand antagonists of A-gliadin 57-73 is an alternative strategy to generate genetically modified wheat that is therapeutic rather than "non-toxic" in coeliac disease.

Example 9

Definition of Core Epitope Sequence:

Comparison of peptides corresponding to truncations of A-gliadin 56-75 from the N- and C-terminal indicated that the core sequence of the T cell epitope is PELPY (A-gliadin 64-68). Attempts to define non-agonists and antagonists will focus on variants of A-gliadin that are substituted at residues that substantially contribute to its bioactivity.

Peptides corresponding to A-gliadin 57-73 QE65 with alanine (Figure 15) or lysine (Figure 16) substituted for residues 57 to 73 were compared in the IFN gamma ELISPOT using peripheral blood mononuclear cells (PBMC) from

coeliac volunteers 6 days after commencing a 3-day gluten challenge (n=8). (BL is blank, E is A-gliadin 57-73 QE65: QLQPFPQPELPYPQPQS (SEQ ID NO:2)).

It was found that residues corresponding to A-gliadin 60-70 QE65 (PFPQPELPYPQ (SEQ ID NO:14)) contribute substantially to the bioactivity in A-gliadin 57-73 QE65. Variants of A-gliadin 57-73 QE65 substituted at positions 60-70 are assessed in a 2-step procedure. Initially, A-gliadin 57-73 QE65 substituted at positions 60-70 using 10 different amino acids with contrasting properties are assessed. A second group of A-gliadin 57-73 QE65 variants (substituted with all other naturally occurring amino acids except cysteine at positions that prove are sensitive to modification) are assessed in a second round.

Example 10

Agonist activity of substituted variants of A-gliadin 57-73 QE65

A-gliadin 60-70 QE65 is the core sequence of the dominant T cell epitope in A-gliadin. Antagonist and non-agonist peptide variants of this epitope are most likely generated by modification of this core sequence. Initially, A-gliadin 57-73 QE65 substituted at positions 60-70 using 10 different amino acids with contrasting properties will be assessed in the IFNgamma ELISPOT using PBMC from coeliac subjects 6 days after starting 3 day gluten challenge. A second group of A-gliadin 57-73 QE65 variants (substituted with all other naturally occurring amino acids except cysteine) at positions 61-70 were also assessed. Both groups of peptides (all at 50 mcg/ml, in duplicate) were assessed using PBMC from 8 subjects and compared to the unmodified peptide (20 replicates per assay). Previous studies indicate that the optimal concentration for A-gliadin 57-73 QE65 in this assay is between 10 and 100 mcg/ml.

Results are expressed as mean response in spot forming cells (95% confidence interval) as % A-G 57-73 QE65 mean response in each individual. Unpaired t-tests will be used to compare ELISPOT responses of modified peptides with A-G 57-73 QE65. Super-agonists were defined as having a greater response than A-G 57-73 QE65 at a level of significance of $p<0.01$; partial

agonists as having a response less than A-G 57-73 QE65 at a level of significance of $p<0.01$, and non-agonists as being not significantly different ($p>0.01$) from blank (buffer without peptide). Peptides with agonist activity 30% or less that of A-gliadin 57-73 QE65 were considered "suitable" partial or non-agonists to assess for antagonistic activity (see Table 8 and Figures 17-27).

The IFNgamma ELISPOT response of PBMC to A-gliadin 57-73 QE65 is highly specific at a molecular level. Proline at position 64 (P64), glutamate at 65 (E65) and leucine at position 66 (L66), and to a lesser extent Q63, P67, Y68 and P69 are particularly sensitive to modification. The substitutions Y61 and Y70 both generate super-agonists with 30% greater bioactivity than the parent peptide, probably by enhancing binding to HLA-DQ2 since the motif for this HLA molecule indicates a preference for bulky hydrophobic resides at positions 1 and 9. Eighteen non-agonist peptides were identified. Bioactivities of the variants (50 mcg/ml): P65, K64, K65 and Y65 (bioactivity 7-8%) were comparable to blank (7%). In total, 57 mutated variants of A-gliadin 57-73 QE65 were 30% or less bioactive than A-gliadin 57-73 QE65.

The molecular specificity of the peripheral blood lymphocyte (PBL) T cell response to the dominant epitope, A-gliadin 57-73 QE65, is consistently reproducible amongst HLA-DQ2+ coeliac subjects, and is highly specific to a restricted number of amino acids in the core 7 amino acids. Certain single-amino acid variants of A-gliadin 57-73 QE65 are consistently non-agonists in all HLA-DQ2+ coeliac subjects.

Example 11

Antagonist activity of substituted variants

The homogeneity of the PBL T cell response to A-gliadin 57-73 QE65 in HLA-DQ2+ coeliac disease suggests that altered peptide ligands (APL) capable of antagonism in PBMC ex vivo may exist, even though the PBL T cell response is likely to be poly- or oligo-clonal. APL antagonists are generally weak agonists. Fifty-seven single amino acid-substituted variants of A-gliadin 57-73 QE65 with agonist activity 30% or less have been identified and are suitable

candidates as APL antagonists. In addition, certain weakly bioactive naturally occurring polymorphisms of A-gliadin 57-73 QE65 have also been identified (see below) and may be "naturally occurring" APL antagonists. It has also been suggested that competition for binding MHC may also antagonise antigen-specific T cell immune. Hence, non-gliadin peptides that do not induce IFNgamma responses in coeliac PBMC after gluten challenge but are known to bind to HLA-DQ2 may be capable of reducing T cell responses elicited by A-gliadin 57-73 QE65. Two peptides that bind avidly to HLA-DQ2 are HLA class 1 α 46-60 (HLA 1a) (PRAPWIEQEGPEYW (SEQ ID NO:15)) and thyroid peroxidase (tp) 632-645Y (IDVWLGGLLAENFLPY (SEQ ID NO:16)).

Simultaneous addition of peptide (50μg/ml) or buffer and A-gliadin 57-73 QE65 (10μg/ml) in IFNgamma ELISPOT using PBMC from coeliac volunteers 6 days after commencing 3 day gluten challenge (n=5). Results were expressed as response with peptide plus A-G 57-73 QE65 (mean of duplicates) as % response with buffer plus A-G 57-73 QE65 (mean of 20 replicates). (See Table 9).

Four single amino acid-substituted variants of A-gliadin 57-73 QE65 reduce the interferon gamma PBMC ELISPOT response to A-gliadin 57-73 QE65 ($p<0.01$) by between 25% and 28%, 13 other peptide variants reduce the ELISPOT response by between 18% and 24% ($p<0.06$). The HLA-DQ2 binder, thyroid peroxidase (tp) 632-645Y reduces PBMC interferon gamma responses to A-gliadin 57-73 QE65 by 31% ($p<0.0001$) but the other HLA-DQ2 binder, HLA class 1 α 46-60, does not alter responses (see Tables 9 and 10). The peptide corresponding to a transglutaminase-modified polymorphism of A-gliadin 57-73, SwissProt accession no.: P04725 82-98 QE90 (PQPQPFPPELPYPQPQS (SEQ ID NO:17)) reduces responses to A-gliadin 57-73 QE65 by 19% ($p<0.009$) (see Table 11).

Interferon gamma responses of PBMC to A-gliadin 57-73 QE65 in ELISPOT assays are reduced by co-administration of certain single-amino acid A-gliadin 57-73 QE65 variants, a polymorphism of A-gliadin 57-73 QE65, and an unrelated peptide known to bind HLA-DQ2 in five-fold excess. These finding suggest that altered peptide ligand antagonists of A-gliadin 57-73 QE65 exist.

Not only putative APL antagonists but also certain peptides that bind HLA-DQ2 effectively reduce PBL T cell responses to A-gliadin 57-73 QE65.

These findings support two strategies to interrupt the T cell response to the dominant A-gliadin epitope in HLA-DQ2+ coeliac disease.

1. Optimisation of APL antagonists by substituting amino acids at more than one position (64-67) for use as "traditional" peptide pharmaceuticals or for specific genetic modification of gliadin genes in wheat.
2. Use of high affinity HLA-DQ2 binding peptides to competitively inhibit presentation of A-gliadin 57-73 QE65 in association with HLA-DQ2.

These two approaches may be mutually compatible. Super-agonists were generated by replacing F61 and Q70 with tyrosine residues. It is likely these super-agonists resulted from improved binding to HLA-DQ2 rather than enhanced contact with the T cell receptor. By combining these modifications with other substitutions that generate modestly effective APL antagonists might substantially enhance the inhibitory effect of substituted A-gliadin 57-73 QE65 variants.

Example 12

Development of interferon gamma ELISpot using PBMC and A-gliadin 57-73 QE65 and P04724 84-100 QE92 as a diagnostic for coeliac disease: Definition of immune-responsiveness in newly diagnosed coeliac disease

Induction of responsiveness to the dominant A-gliadin T cell epitope in PBMC measured in the interferon gamma ELISpot follows gluten challenge in almost all DQ2+ coeliac subjects following a long term strict gluten free diet (GFD) but not in healthy DQ2+ subjects after 4 weeks following a strict GFD. A-gliadin 57-73 QE65 responses are not measurable in PBMC of coeliac subjects before gluten challenge and pilot data have suggested these responses could not be measured in PBMC of untreated coeliacs. These data suggest that in coeliac disease immune-responsiveness to A-gliadin 57-73 QE65 is restored following antigen exclusion (GFD). If a diagnostic test is to be developed using the

ELISpot assay and PBMC, it is desirable to define the duration of GFD required before gluten challenge is capable of inducing responses to A-gliadin 57-73 QE65 and other immunoreactive gliadin peptides in blood.

Newly diagnosed DQ2+ coeliac subjects were recruited from the gastroenterology outpatient service. PBMC were prepared and tested in interferon gamma ELISpot assays before subjects commenced GFD, and at one or two weeks after commencing GFD. In addition, gluten challenge (3 days consuming 4 slices standard white bread, 200g/day) was performed at one or two weeks after starting GFD. PBMC were prepared and assayed on day six are after commencing gluten challenge. A-gliadin 57-73 QE65 (A), P04724 84-100 QE92 (B) (alone and combined) and A-gliadin 57-73 QP65 (P65) (non-bioactive variant, see above) (all 25 mcg/ml) were assessed.

All but one newly diagnosed coeliac patient was DQ2+ (one was DQ8+) (n=11). PBMC from newly diagnosed coeliacs that were untreated, or after 1 or 2 weeks following GFD did not show responses to A-gliadin 57-73 QE65 and P04724 84-100 QE92 (alone or combined) that were not significantly different from blank or A-gliadin 57-73 QP65 (n=9) (see Figure 28). Gluten challenge in coeliacs who had followed GFD for only one week did not substantially enhance responses to A-gliadin 57-73 QE65 or P04724 84-100 QE92 (alone or combined). But gluten challenge 2 weeks after commencing GFD did induce responses to A-gliadin 57-73 QE65 and P04724 84-100 QE92 (alone or combined) that were significantly greater than the non-bioactive variant A-gliadin 57-73 QP65 and blank. Although these responses after gluten challenge at 2 weeks were substantial they appear to be less than in subjects >2 months after commencing GFD. Responses to A-gliadin 57-73 QE65 alone were equivalent or greater than responses to P04724 84-100 QE92 alone or when mixed with A-gliadin 57-73 QE65. None of the subjects experienced troubling symptoms with gluten challenge.

Immune responsiveness (as measured in PBMC after gluten challenge) to A-gliadin is partially restored 2 weeks after commencing GFD, implying that "immune unresponsiveness" to this dominant T cell epitope prevails in untreated

coeliac disease and for at least one week after starting GFD. The optimal timing of a diagnostic test for coeliac disease using gluten challenge and measurement of responses to A-gliadin 57-73 QE65 in the ELISpot assay is at least 2 weeks after commencing a GFD.

Interferon gamma-secreting T cells specific to A-gliadin 57-73 QE65 cannot be measured in the peripheral blood in untreated coeliacs, and can only be induced by gluten challenge after at least 2 weeks GFD (antigen exclusion). Therefore, timing of a diagnostic test using this methodology is crucial and further studies are needed for its optimization. These finding are consistent with functional anergy of T cells specific for the dominant epitope, A-gliadin 57-73 QE65, reversed by antigen exclusion (GFD). This phenomenon has not been previously demonstrated in a human disease, and supports the possibility that T cell anergy may be inducible with peptide therapy in coeliac disease.

Example 13

Comprehensive Mapping of Wheat Gliadin T Cell Epitopes

Antigen challenge induces antigen-specific T cells in peripheral blood. In coeliac disease, gluten is the antigen that maintains this immune-mediated disease. Gluten challenge in coeliac disease being treated with a gluten free diet leads to the appearance of gluten-specific T cells in peripheral blood, so enabling determination of the molecular specificity of gluten T cell epitopes. As described above, we have identified a single dominant T cell epitope in a model gluten protein, A-gliadin (57-73 deamidated at Q65). In this Example, gluten challenge in coeliac patients was used to test all potential 12 amino acid sequences in every known wheat gliadin protein derived from 111 entries in Genbank. In total, 652 20mer peptides were tested in HLA-DQ2 and HLA-DQ8 associated coeliac disease. Seven of the 9 coeliac subjects with the classical HLA-DQ2 complex (HLA-DQA1*05, HLA-DQB1*02) present in over 90% of coeliacs had an inducible A-gliadin 57-73 QE65- and gliadin-specific T cell response in peripheral blood. A-gliadin 57-73 was the only significant α-gliadin T cell epitope, as well as the most potent gliadin T cell epitope, in HLA-DQ2-associated

coeliac disease. In addition, there were as many as 5 families of structurally related peptides that were between 10 and 70% as potent as A-gliadin 57-73 in the interferon-γ ELISpot assay. These new T cell epitopes were derived from γ- and ω-gliadins and included common sequences that were structurally very similar, but not identical to the core sequence of A-gliadin 57-73 (core sequence: FPQPQLPYP (SEQ ID NO:18)), for example: FPQPQQPFP (SEQ ID NO:19) and PQQPQQPFP (SEQ ID NO:20). Although no homologues of A-gliadin 57-73 have been found in rye or barley, the other two cereals toxic in coeliac disease, the newly defined T cell epitopes in γ- and ω-gliadins have exact matches in rye and barley storage proteins (secalins and hordeins, respectively).

Coeliac disease not associated with HLA-DQ2 is almost always associated with HLA-DQ8. None of the seven HLA-DQ8+ coeliac subjects had inducible A-gliadin 57-73-specific T cell responses following gluten challenge, unless they also possessed the complete HLA-DQ2 complex. Two of 4 HLA-DQ8+ coeliac subjects who did not possess the complete HLA-DQ2 complex, had inducible gliadin peptide-specific T cell responses following gluten challenge. In one HLA-DQ8 subject, a novel dominant T cell epitope was identified with the core sequence LQPQNPSQQQPQ (SEQ ID NO:21). The transglutaminase-deamidated version of this peptide was more potent than the non-deamidated peptide. Previous studies suggest that the transglutaminase-deamidated peptide would have the sequence LQPENPSQEQPE (SEQ ID NO:22); but further studies are required to confirm this sequence. Amongst the healthy HLA-DQ2 (10) and HLA-DQ8 (1) subjects who followed a gluten free diet for a month, gliadin peptide-specific T cell responses were uncommon, seldom changed with gluten challenge, and were never potent T cell epitopes revealed with gluten challenge in coeliac subjects. In conclusion, there are unlikely to be more than six important T cell epitopes in HLA-DQ2-associated coeliac disease, of which A-gliadin 57-73 is the most potent. HLA-DQ2- and HLA-DQ8-associated coeliac disease do not share the same T cell specificity.

We have shown that short-term gluten challenge of individuals with coeliac disease following a gluten free diet induces gliadin-specific T cells in

peripheral blood. The frequency of these T cells is maximal in peripheral blood on day 6 and then rapidly wanes over the following week. Peripheral blood gliadin-specific T cells express the integrin α4β7 that is associated with homing to the gut lamina propria. We exploited this human antigen-challenge design to map T cell epitopes relevant to coeliac disease in the archetypal gluten α-gliadin protein, A-gliadin. Using 15mer peptides overlapping by 10 amino acids with and without deamidation by transglutaminase (tTG), we demonstrated that T cells induced in peripheral blood initially target only one A-gliadin peptide, residues 57-73 in which glutamine at position 65 is deamidated. The epitope is HLA-DQ2-restricted, consistent with the intimate association of coeliac disease with HLA-DQ2.

Coeliac disease is reactivated by wheat, rye and barley exposure. The α/β-gliadin fraction of wheat gluten is consistently toxic in coeliac disease, and most studies have focused on these proteins. The gene cluster coding for α/β-gliadins is located on wheat chromosome 6C. There are no homologues of α/β-gliadins in rye or barley. However, all three of the wheat gliadin subtypes (α/β, γ, and ω) are toxic in coeliac disease. The γ- and ω-gliadin genes are located on chromosome 1A in wheat, and are homologous to the secalins and hordeins in rye and barley.

There are now genes identified for 61 α-gliadins in wheat (Triticum aestivum). The α-gliadin sequences are closely homologous, but the dominant epitope in A-gliadin derives from the most polymorphic region in the α-gliadin sequence. Anderson et al (1997) have estimated that there are a total of about 150 distinct α-gliadin genes in T. aestivum, but many are psuedogenes. Hence, it is unlikely that T-cell epitopes relevant to coeliac disease are not included within known α-gliadin sequences.

Our work has identified a group of deamidated α-gliadin peptides almost identical to A-gliadin 57-73 as potent T cell epitopes specific to coeliac disease. Over 90% of coeliac patients are HLA-DQ2+, and so far, we have only assessed HLA-DQ2+ coeliac subjects after gluten challenge. However, coeliac patients who do not express HLA-DQ2 nearly all carry HLA-DQ8. Hence, it is critical to

know whether A-gliadin 57-73 and its homologues in other wheat, rye and barley gluten proteins are the only T-cell epitopes recognized by T cells induced by gluten challenge in both HLA-DQ2+ and HLA-DQ8+ coeliac disease. If this were the case, design of peptide therapeutics for coeliac disease might only require one peptide.

Homologues of A-gliadin 57-73 as T-cell epitopes

Initial searches of SwissProt and Trembl gene databases for cereal genes coding for the core sequence of A-gliadin 57-73 (PQLPY <SEQ ID NO:12>) only revealed α/β-gliadins. However, our fine-mapping studies of the A-gliadin 57-73 QE65 epitope revealed a limited number of permissive point substitutions in the core region (PQLP) (note Q65 is actually deamidated in the epitope). Hence, we extended our search to genes in SwissProt or Trembl databases encoding for peptides with the sequence XXXXXXXPQ[ILMP][PST]XXXXXX (SEQ ID NO:23). Homologues were identified amongst γ-gliadins, glutenins, hordeins and secalins (see Table 12). A further homologue was identified in ω-gliadin by visual search of the three ω-gliadin entries in Genbank.

These homologues of A-gliadin 57-73 were assessed after deamidation by tTG (or synthesis of the glutamate(QE)-substituted variant in four close homologues) using the IFNγ ELISpot assay with peripheral blood mononuclear cells after gluten challenge in coeliac subjects. The ω-gliadin sequence (AAG17702 141-157) was the only bioactive peptide, approximately half as potent as A-gliadin 57-73 (see Table 12, and Figure 29). Hence, searches for homologues of the dominant A-gliadin epitope failed to account for the toxicity of γ-gliadin, secalins, and hordeins.

Methods

Design of a set of peptides spanning all possible wheat gliadin T-cell epitopes

In order to identify all possible T cell epitopes coded by the known wheat (Triticum aestivum) gliadin genes or gene fragments (61 α/β-, 47 γ-, and 3 ω-gliadin entries in Genbank), gene-derived protein sequences were aligned using the CustalW software (MegAlign) and arranged into phylogenetic groupings (see

Table 22). Many entries represented truncations of longer sequences, and many gene segments were identical except for the length of polyglutamine repeats or rare substitutions. Hence, it was possible to rationalize all potential unique 12 amino acid sequences encoded by known wheat genes to be included in a set of 652 20mer peptides. (Signal peptide sequences were not included). Peptide sequences are listed in Table 23.

Comprehensive epitope mapping

Healthy controls (HLA-DQ2+ n=10, and HLA-DQ8+ n=1) who had followed a gluten free diet for 4 weeks, and coeliac subjects (six HLA-DQ2, four complex heterozygotes HLA-DQ2/8, and three HLA-DQ8/X) (see Table 13) following long-term gluten free diet were studied before and on day 6 and 7 after 3-day gluten challenge (four 50g slices of standard white bread – Sainsbury's sandwich bread, each day). Peripheral blood (a total of 300ml over seven days) was collected and peripheral blood mononuclear cells (PBMC) were separated by Lymphoprep density gradient. PBMC were incubated with pools of 6 or 8 20mer peptides, or single peptides with or without deamidation by tTG in overnight interferon gamma (IFNγ) ELISpot assays.

Peptides were synthesized in batches of 96 as Pepsets (Mimotopes Inc., Melbourne Australia). Approximately 0.6 micromole of each of 652 20mers was provided. Two marker 20mer peptides were included in each set of 96 (VLQQHNIAHGSSQVLQESTY – peptide 161 (SEQ ID NO:24), and IKDFHVYFRESRDALWKGPG (SEQ ID NO:25)) and were characterized by reverse phase-HPLC and amino acid sequence analysis. Average purities of these marker peptides were 50% and 19%, respectively. Peptides were initially dissolved in acetonitrile (10%) and Hepes 100mM to 10mg/ml.

The final concentration of individual peptides in pools (or alone) incubated with PBMC for the IFNγ ELISpot assays was 20 μg/ml. Five-times concentrated solutions of peptides and pools in PBS with calcium chloride 1mM were aliquotted and stored in 96-well plates according to the template later used in ELISpot assays. Deamidated peptides and pools of peptides were prepared by incubation with guinea pig tissue tTG (Sigma T5398) in the ratio 100:32 μg/ml

for two hours at 37°C. Peptides solutions were stored at –20°C and freshly thawed prior to use.

Gliadin (Sigma G3375) (100 mg/ml) in endotoxin-free water and 2M urea was boiled for 10 minutes, cooled to room temperature and incubated with filter (0.2 μm)-sterilised pepsin (Sigma P6887) (2 mg/ml) in HCl 0.02M or chymotrypsin (C3142) (4mg/ml) in ammonium bicarbonate (0.2M). After incubation for 4 hours, pepsin-digested gliadin was neutralized with sodium hydroxide, and then both pepsin- and chymotrypsin-digested gliadin were boiled for 15 minutes. Identical incubations with protease in which gliadin was omitted were also performed. Samples were centrifuged at 15 000g, then protein concentrations were estimated in supernatants by the BCA method (Pierce, USA). Before final use in IFNγ ELISpot assays, aliquots of gliadin-protease were incubated with tTG in the ratio 2500:64 μg/ml.

IFNγ ELISpot assays (Mabtech, Sweden) were performed in 96-well plates (MAIP S-45, Millipore) in which each well contained 25μl of peptide solution and 100μl of PBMC (2-8x10^5/well) in RPMI containing 10% heat inactivated human AB serum. Deamidated peptide pools were assessed in one 96-well ELISpot plate, and peptides pools without deamidation in a second plate (with an identical layout) on both day 0 and day 6. All wells in the plate containing deamidated peptides included tTG (64 μg/ml). In each ELISpot plate there were 83 wells with peptide pools (one unique pool in each well), and a series of wells for "control" peptides (peptides all >90% purity, characterized by MS and HPLC, Research Genetics): P04722 77-93 (QLQPFPQPQLPYPQPQP (SEQ ID NO:26)), P04722 77-93 QE85 (in duplicate) (QLQPFPQPELPYPQPQP (SEQ ID NO:27)), P02863 77-93 (QLQPFPQPQLPYSQPQP (SEQ ID NO:28)), P02863 77-93 QE85 (QLQPFPQPELPYSQPQP (SEQ ID NO:29)), and chymotrypsin-digested gliadin (500 μg/ml), pepsin-digested gliadin (500 μg/ml), chymotrypsin (20 μg/ml) alone, pepsin (10 μg/ml) alone, and blank (PBS+/-tTG) (in triplicate).

After development and drying, IFNγ ELISpot plates were assessed using the MAIP automated ELISpot plate counter. In HLA-DQ2 healthy and coeliac

subjects, induction of spot forming cells (sfc) by peptide pools in the IFNγ ELISpot assay was tested using a one-tailed Wilcoxon Matched-Pairs Signed-Ranks test (using SPSS software) applied to spot forming cells (sfc) per million PBMC minus blank on day 6 versus day 0 ("net response"). Significant induction of an IFNγ response to peptide pools in PBMC by *in vivo* gluten challenge was defined as a median "net response" of at least 10 sfc/million PBMC and $p<0.05$ level of significance. Significant response to a particular pool of peptides on day 6 was followed by assessment of individual peptides within each pool using PBMC drawn the same day or on day 7.

For IFNγ ELISpot assays of individual peptides, bioactivity was expressed as a percent of response to P04722 77-93 QE85 assessed in the same ELISpot plate. Median response to blank (PBS alone) was 0.2 (range 0-5) sfc per well, and the positive control (P04722 77-93 QE85) 76.5 (range: 25-282) sfc per well using a median of 0.36 million (range: 0.3-0.72) PBMC. Hence, median response to blank expressed as a percentage of P04722 77-93 QE65 was 0.2% (range: 0-6.7). Individual peptides with mean bioactivity greater than10% that of P04722 QE85 were analyzed for common structural motifs.

Results

Healthy HLA-DQ2 subjects

None of the healthy HLA-DQ2+ subjects following a gluten free diet for a month had IFNγ ELISpot responses to homologues of A-gliadin 57-73 before or after gluten challenge. However, in 9/10 healthy subjects, gluten challenge was associated with a significant increase in IFNγ responses to both peptic- and chymotryptic-digests of gliadin, from a median of 0-4 sfc/million on day 0 to a median of 16-29 sfc/million (see Table 14). Gliadin responses in healthy subjects were unaffected by deamidation (see Table 15). Amongst healthy subjects, there was no consistent induction of IFNγ responses to specific gliadin peptide pools with gluten challenge (see Figure 30, and Table 16). IFNγ ELISpot responses were occasionally found, but these were weak, and not altered by deamidation. Many of the strongest responses to pools were also present on day 0 (see Table 17, subjects H2, H8 and H9). Four healthy subjects did show definite responses

to pool 50,and the two with strongest responses on day 6 also had responses on day 0. In both subjects, the post-challenge responses to pool 50 responses were due to peptide 390 (QQTYPQRPQQPFPQTQQPQQ (SEQ ID NO:30)).

HLA-DQ2 coeliac subjects

Following gluten challenge in HLA-DQ2+ coeliac subjects, median IFNγ ELISpot responses to P04722 77-93 E85 rose from a median of 0 to 133 sfc/million (see Table 4). One of the six coeliac subjects (C06) did not respond to P04722 77-93 QE85 (2 sfc/million) and had only weak responses to gliadin peptide pools (maximum: Pool 50+tTG 27 sfc/million). Consistent with earlier work, bioactivity of wild-type P04722 increased 6.5 times with deamidation by tTG (see Table 15). Interferon-gamma responses to gliadin-digests were present at baseline, but were substantially increased by gluten challenge from a median of 20 up to 92 sfc/million for chymotryptic-gliadin, and from 44 up to 176 sfc/million for peptide-gliadin. Deamidation of gliadin increased bioactivity by a median of 3.2 times for chymotryptic-gliadin and 1.9 times for peptic-gliadin (see Table 15). (Note that the acidity required for digestion by pepsin is likely to result in partial deamidation of gliadin.)

In contrast to healthy subjects, gluten challenge induced IFNγ ELISpot responses to 22 of the 83 tTG-treated pools including peptides from α-, γ- and ω-gliadins (see Figure 31, and Table 17). Bioactivity of pools was highly consistent between subjects (see Table 18). IFNγ ELISpot responses elicited by peptide pools were almost always increased by deamidation (see Table 17). But enhancement of bioactivity of pools by deamidation was not as marked as for P04722 77-73 Q85, even for pools including homologues of A-gliadin 57-73. This suggests that Pepset peptides were partially deamidated during synthesis or in preparation, for example the Pepset peptides are delivered as salts of trifluoracetic acid (TFA) after lyophilisation from a TFA solution.

One hundred and seventy individual tTG-deamidated peptides from 21 of the most bioactive pools were separately assessed. Seventy-two deamidated peptides were greater than 10% as bioactive as P04722 77-93 QE85 at an equivalent concentration (20 μg/ml) (see Table 19). The five most potent

peptides (85-94% bioactivity of P04722 QE85) were previously identified α-gliadin homologues A-gliadin 57-73. Fifty of the bioactive peptides were not homologues of A-gliadin 57-73, but could be divided into six families of structurally related sequences (see Table 20). The most bioactive sequence of each of the peptide families were: PQQPQQPQQPFPQPQQPFPW (SEQ ID NO:31) (peptide 626, median 72% bioactivity of P04722 QE85), QQPQQPFPQPQQPQLPFPQQ (SEQ ID NO:32) (343, 34%), QAFPQPQQTFPHQPQQQFPQ (SEQ ID NO:33) (355, 27%), TQQPQQPFPQQPQQPFPQTQ (SEQ ID NO:34) (396, 23%), PIQPQQPFPQQPQQPQQPFP (SEQ ID NO:35) (625, 22%), PQQSFSYQQQPFPQQPYPQQ (SEQ ID NO:36) (618, 18%) (core sequences are underlined). All of these sequences include glutamine residues predicted to be susceptible to deamidation by transglutaminase (e.g. QXP, QXPF (SEQ ID NO:37), QXX[FY] (SEQ ID NO:38)) (see Vader et al 2002). Some bioactive peptides contain two core sequences from different families.

Consistent with the possibility that different T-cell populations respond to peptides with distinct core sequences, bioactivity of peptides from different families appear to be additive. For example, median bioactivity of tTG-treated Pool 81 was 141% of P04722 QE85, while bioactivity of individual peptides was in rank order: Peptide 631 (homologue of A-gliadin 57-73) 61%, 636 (homologue of 626) 51%, and 635 19%, 629 16%, and 634 13% (all homologues of 396).

Although likely to be an oversimplification, the contribution of each "peptide family" to the summed IFNγ ELISpot response to gliadin peptides was compared in the HLA-DQ2+ coeliac subjects (see Figure 32). Accordingly, the contribution of P04722 77-73 E85 to the summed response to gliadin peptides is between 1/5 and 2/3.

Using the peptide homology search programme, WWW PepPepSearch, which can be accessed through the world wide web of the internet at, for example, "cbrg.inf.ethz.ch/subsection3_1_5.html.", and by direct comparison with Genbank sequences for rye secalins, exact matches were found for the core sequences QQPFPQPQQPFP (SEQ ID NO:39) in barley hordeins (HOR8) and

rye secalins (A23277, CAA26449, AAG35598), QQPFPQQPQQPFP (SEQ ID NO:40) in barley hordeins (HOG1 and HOR8), and for PIQPQQPFPQQP (SEQ ID NO:41) also in barley hordeins (HOR8).

HLA-DQ8-associated coeliac disease

Seven HLA-DQ8+ coeliac subjects were studied before and after gluten challenge. Five of these HLA-DQ8+ (HLA-DQA0*0301-3, HLA-DQB0*0302) subjects also carried one or both of the coeliac disease-associated HLA-DQ2 complex (DQA0*05, DQB0*02). Two of the three subjects with both coeliac-associated HLA-DQ complexes had potent responses to gliadin peptide pools (and individual peptides including P04722 77-93 E85) that were qualitatively and quantitatively identical to HLA-DQ2 coeliac subjects (see Figures 33 and 34, and Table 18). Deamidated peptide pool 74 was bioactive in both HLA-DQ2/8 subjects, but only in one of the 6 HLA-DQ2/X subjects. Pretreatment of pool 74 with tTG enhances bioactivity between 3.8 and 22-times, and bioactivity of tTG-treated pool 74 in the three responders is equivalent to between 78% and 350% the bioactivity of P04722 77-93 E85. Currently, it is not known which peptides are bioactive in Pool 74 in subject C02, C07, and C08.

Two of the four HLA-DQ8 coeliac subjects that lacked both or one of the HLA-DQ2 alleles associated with coeliac disease showed very weak IFNγ ELISpot responses to gliadin peptide pools, but the other two did respond to both protease-digested gliadin and specific peptide pools. Subject C12 (HLA-DQ7/8) responded vigorously to deamidated Pools 1-3 (see Figure 35). Assessment of individual peptides in these pools identified a series of closely related bioactive peptides including the core sequence LQPQNPSQQQPQ (SEQ ID NO:42) (see Table 20). Previous work (by us) has demonstrated that three glutamine residues in this sequence are susceptible to tTG-mediated deamidation (underlined). Homology searches using WWW PepPepSearch have identified close matches to LQPQNPSQQQPQ (SEQ ID NO:43) only in wheat α-gliadins.

The fourth HLA-DQ8 subject (C11) had inducible IFNγ ELISpot responses to tTG-treated Pool 33 (see Figure 36). Pools 32 and 33 include

polymorphisms of a previously defined HLA-DQ8 restricted gliadin epitope (QQYPSGQGSFQPSQQNPQ (SEQ ID NO:44)) active after deamidation by tTG (underlined Gln are deamidated and convey bioactivity) (van der Wal et al 1998). Currently, it is not known which peptides are bioactive in Pool 33 in subject C11.

Comprehensive T cell epitope mapping in HLA-DQ2-associated coeliac disease using in vivo gluten challenge and a set of 652 peptides spanning all known 12 amino acid sequences in wheat gliadin has thus identified at least 72 peptides at 10% as bioactive as the known α-gliadin epitope, A-gliadin 57-73 E65. However, these bioactive peptides can be reduced to a set of perhaps as few as 5 distinct but closely related families of peptides. Almost all these peptides are rich in proline, glutamine, phenylalanine, and/or tyrosine and include the sequence PQ(QL)P(FY)P (SEQ ID NO:45). This sequence facilitates deamidation of Q in position 2 by tTG. By analogy with deamidation of A-gliadin 57-68 (Arentz-Hansen 2000), the enhanced bioactivity of these peptides generally found with deamidation by tTG may be due to increased affinity of binding for HLA-DQ2.

Cross-reactivity amongst T cells in vivo recognizing more than one of these bioactive gliadin peptides is possible. However, if each set of related peptides does activate a distinct T cell population in vivo, the epitope corresponding to A-gliadin 57-73 E65 is the most potent and is generally recognized by at least 40% of the peripheral blood T cells that secrete IFNγ in response to gliadin after gluten challenge.

No gliadin-peptide specific responses were found in HLA-DQ2/8 coeliac disease that differed qualitatively from those in HLA-DQ2/*X*-associated coeliac disease. However, peripheral blood T cells in HLA-DQ8+ coeliac subjects without both HLA-DQ2 alleles did not recognize A-gliadin 57-73 E65 homologues. Two different epitopes were dominant in two HLA-DQ8+ coeliacs. The dominant epitope in one of these HLA-DQ8+ individuals has not been identified previously (LQPQNPSQQQPQ (SEQ ID NO:46)).

Given the teaching herein, design of an immunotherapy for coeliac disease utilizing all the commonly recognised T cell epitopes is practical and may

include fewer than six distinct peptides. Epitopes in wheat γ- and ω-gliadins are also present in barley hordeins and rye secalins.

Example 14

Several ELISpot assays were performed as previously described and yielded the following results and/or conclusions:

Examination of multiple α-gliadin polymorphisms with PQLPY

Potent agonists of A-gliadin 57-73QE (G01) include

QLQPFPQPELPYPQPQS (G01) (SEQ ID NO:2),

PQL-Y----------P (G10), and

PQPQPFL---------- (G12).

Less potent include

----------L------P (G04),

----------R----------P (G05), and

----------S------P (G06).

Less potent yet include

--------L----------S------P (G07),

--------S----------S------P (G08),

----------S--S------P (G09), and

PQPQPFP---------- (G13).

Dashes indicate identity with the G01 sequence in the particular position.

Gluten challenge induces A-gliadin 57-73 QE65 T cells only after two weeks of gluten-free diet in newly diagnosed coeliac disease

Additional analyses indicated that tTG-deamidated gliadin responses change after two weeks of gluten-free diet in newly diagnosed coeliac disease. Other analyses indicated that deamidated gliadin-specific T cells are $CD4^+\alpha_4\beta_7^+$ HLA-DQ2 restricted.

Optimal epitope (clones versus gluten challenge)

A "dominant" epitope is defined by γIFN ELISpot after gluten challenge. QLQPFPQPELPYPQPQS (SEQ ID NO:2) (100% ELISpot response). Epitopes defined by intestinal T cell clones: QLQPFPQPELPY (SEQ ID NO:13) (27%), PQPELPYPQPELPY (SEQ ID NO:47) (52%), and QQLPQPEQPQQSFPEQERPF (SEQ ID NO:48)(9%).

Dominance among individual peptide responses

Dominance depends on wheat or rye. For wheat, dominant peptides include peptide numbers 89, 90 and 91 (referring to sequence numbers in Table 23). For rye, dominant peptides include peptide numbers 368, 369, 370, 371, and 372 (referring to sequence numbers in Table 23). Some peptides, including 635 and 636 (referring to sequence numbers in Table 23) showed activity in both rye and wheat.

In vivo gluten challenge allows T cell epitope hierarchy to be defined for coeliac disease

The epitope hierarchy is consistent among HLA-DQ2^{+} coeliacs but different for HLA-DQ8^{+} coeliacs. The hierarchy depends on what cereal is consumed. Deamidation generates almost all gliadin epitopes. HLA-DQ2, DQ8, and DR4 present deamidated peptides. HLA-DQ2/8-associated coeliac disease preferentially present DQ2-associated gliadin epitopes. Gliadin epitopes are sufficiently restricted to justify development of epitope-based therapeutics.

Other analyses indicated the following: HLA-DR3-DQ2 (85-95%) and HLA-DR4-DQ8 (5-15%).

Other analyses indicated the following:

HLA-DQ	HLA-DQA1 allele	HLA-DQB1 allele	Duodenal histology	Gluten free	EMA on gluten

						(on GFD)
C01	2,6	102/6, 501	201, 602	SVA	1 yr	+(-)
C02	2,2	501	201	SVA	1 yr	+(-)
C03	2,5	101/4/5, 501	201, 501	PVA	1 yr	+(-)
C04	2,5	101/4/5, 501	201, 501	SVA	7 yr	+(-)
C05	2,2 +(ND)	201, 501	201, 202	SVA	4 mo	
C06	2,2	201, 501	201, 202	SVA	2 yr	+(-)
C07	2,8	301-3, 501	201, 302	SVA	1 yr	+(-)
C08	2,8	301-3, 501	201,302/8	SVA	11 yr	ND (-)
C09	2,8	301-3, 501	201, 302	SVA	29 yr	+(-)
C10	2,8	201, 301-3	202, 302	IEL	1 yr	+(-)
C11	6,8	102/6, 301-3	602/15, 302/8	IEL	9 mo	- (ND)
C12	8,7	301-3, 505	302, 301/9-10	SVA	2 yr	- (-)
C13	8,8	301	302	SVA	1 yr	+ (+)

Another analysis was carried out to determine the bioactivity of individual tTG-deamidated peptides in pools 1-3 in subject C12. The results are as follows (sequence numbers refer to the peptides listed in Table 23): Sequence 8 (100%), Sequence 5 (85%), Sequence 6 (82%), Sequence 3 (77%), Sequence 1 (67%), Sequence 2 (59%), Sequence 9 (49%), Sequence 7 (49%), Sequence 10 (33%), Sequence 4 (15%), Sequence 12 (8%), Sequence 11 (0%), Sequence 23 (26%), Sequence 14 (18%), Sequence 15 (18%), Sequence 17 (18%), Sequence 16 (13%), Sequence 14 (8%), Sequence 22 (5%), Sequence 18 (3%), Sequence 19 (3%), Sequence 20 (0%), Sequence 21 (0%). The predicted deamidated sequence is LQPENPSQEQPE(SEQ ID NO:22).

Individual ELISpot responses by PBMC (Spot forming cells determined by ELISpot Reader)

Peptide (see Table 23)	C01	C02	C03	C04	C05
65	16	2	1	2	3
66	32	6	13	0	6
67	16	3	4	0	4
68	25	8	4	2	2
69	4	0	0	0	0
70	2	1	0	0	0
71	1	1	0	0	1
72	0	0	0	0	0
73	95	21	42	31	31
74	122	15	29	21	28
75	5	1	2	2	5
76	108	13	28	16	22
77	3	0	1	0	1
78	21	2	3	5	3
79	20	0	2	0	2
80	5	2	0	0	3
81	4	1	2	3	1
82	3	3	5	2	2
83	14	2	0	0	1
84	3	0	0	0	0
85	14	1	2	1	2
86	11	0	2	0	2

Cross-reactivity

To deal with data from 652 peptides in 29 subjects, or to determine when a particular response is a true positive peptide-specific T-cell response, or to determine when a response to a peptide is due to cross-reactivity with another structurally related peptide, expression of a particular peptide response can be as

a percentage of a "dominant" peptide response. Alternately, the expression can be a "relatedness" as correlation coefficients between peptide responses, or via bioinformatics.

Additional epitopes

A representative result is as follows:

Combination of peptides with P04722E (all 20mcg/ml) (n=4)

	Alone	P04722E+
Pep 626	60	135
P04722E	100	110
HLAa	0	85

(expressed as percent P04722E)

626+tT: PQQPQQPQQPFPQPQQPFPW (SEQ ID NO:31)

P04724E: QLQPFPQPELPYPQPQL (SEQ ID NO:49)

TTG-deamidation of peptide 626 (n=12)

No tTG = 100%

TTG = 170%

Substitution at particular positions

Substitution of Peptide 626 PQQP[Q1]QP[Q2]QPFPQP[Q3]QPFPV (n=12)

	Glu	Arg
Q1	95	90
Q2	145	80
Q3	155	10

(expressed as percent wild-type peptide)

Bioactivity of tTG-treated 15mers spanning Peptide 626/627

(PQQPQQPQQPFPQPQQPFPWQP(SEQ ID NO:31)) (n=8)

P1-15	5

P2-16	4
P3-17	3
P4-18	38
P5-19	65
P6-20	95
P7-21	65
P8-22	90

(expressed as percent of maximal 15mer response)

Multiple epitopes:

P04724E: QLQPFPQPQLPYPQPQL (SEQ ID NO:51)

626+tTG: PQQPQQPQQPFPQPQQPFPW (SEQ ID NO:31)

Minimal epitope: QPQQPFPQPQQPFPW (SEQ ID NO:52)

Immunomagnetic depletion of PBMC by beads coated with anti-CD4 and by anti-integrin β_7 depleted IFNγ ELISpot responses, while immunomagnetic depletion of PBMC by beads coated with anti-CD8 or anti-alphaE integrin. Thus, the PBMC secreting IFNγ are CD4+ and $\alpha_4\beta_7$+, associated with homing to the lamina propria in the gut.

Blocked by anti-DQ antibody but not by anti-DR antibody in heterozygotes and homozygotes for HLA-DQ2. This may imply multiple epitopes within one sequence.

T cell epitopes in coeliac disease

Other investigators have characterized certain intestinal T cell clone epitopes. See, e.g., Vader et al., Gastroenterology 2002, 122:1729-37; Arentz-Hansen et al., Gastroenterology 2002, 123:803-809. These are examples of epitopes whose relevance is at best unclear because of the in vitro techniques used to clone T cells.

Intestinal versus peripheral blood clones

Intestinal: 1) intestinal biopsies, 2) T cell clones raised against peptic-tryptic digest of gluten, 3) all HLA-DQ2 restricted, 4) clones respond to gliadin deamidated by transglutaminase.
Peripheral blood: 1) T cell clones raised against gluten are HLA-DR, DQ and DP restricted. Result: Intestinal T cell clones can be exclusively used to map coeliac disease associated epitopes

GDA_9Wheat 307 aa Definition Alpha/Beta-Gliadin MM1 Precursor (Prolamin) Accession P18573 -- Genbank (which is incorporated herein by reference in its entirety)

Intestinal T cell clone epitopes

A definition of intestinal T cell clone epitopes can be found in, for example, Arentz-Hansen et al., J Exp Med. 2000, 191:603-12. Also disclosed therein are gliadin epitopes for intestinal T cell clones. Deamidated QLQPFPQPQLPY(SEQ ID NO:53) is an epitope, with a deamidated sequence of QLQPFPQPELPY(SEQ ID NO:13). There is an HLA-DQ2 restriction. A homology search shows other bioactive rAlpha-gliadins include PQPQLPY(SEQ ID NO:4) singly or duplicated. A majority of T cell clones respond to either/or DQ2-αI: QLQPFPQPELPY(SEQ ID NO:13) DQ2-αII: PQPELPYPQPELPY(SEQ ID NO:47)

Dominant gliadin T cell epitopes

All deamidated by transglutaminase.
Peripheral blood day 6 after gluten challenge: A-gliadin 57-73: QLQPFPQPELPYPQPQS(SEQ ID NO:2)
Intestinal T cell clones: DQ2-αI: QLQPFPQPELPY(SEQ ID NO:13) DQ2-αII: PQPELPYPQPELPY(SEQ ID NO:47)

Intestinal T-cell Clone Epitope Mapping

α-Gliadins	A1	PFPQPQLPY(SEQ ID NO:54)

		A2	PQPQLPYPQ(SEQ ID NO:55)
		A3	PYPQPQLPY(SEQ ID NO:56)
		Glia-20	PQQPYPQPQPQ(SEQ ID NO:57)
	Γ-Gliadins	G1	PQQSFPQQQ(SEQ ID NO:58)
		G2	IIPQQPAQ(SEQ ID NO:59)
		G3	FPQQPQQPYPQQP(SEQ ID NO:60)
		G4	FSQPQQQFPQPQ(SEQ ID NO:61)
		G5	LQPQQPFPQQPQQPYPQQPQ(SEQ ID NO:62)
		Glu-21	QSEQSQQPFPQQF(SEQ ID NO:63)
		Glu-5	Q(IL)PQQPQQF(SEQ ID NO:64)
	Glutenin	Glt-156	PFSQQQQSPF(SEQ ID NO:65)
		Glt-17	PFSQQQQQ(SEQ ID NO:66)

Gluten exposure and induction of IFNγ-secreting A-Gliadin 57-73QE65-specific T cells in peripheral blood

Untreated coeliac disease, followed by gluten free diet for 1, 2, or 8 weeks, followed by gluten exposure (3 days bread 200g/day), followed by gluten free diet

Result 1: Duration of gluten free diet and IFNγ ELISpot responses on day 0 and day 6 of gluten challenge: A-gliadin 57-73 QE65 (results expressed as IFNγ specific spots/million PPBMC)

Day 0: none (5), 1 week (1), 2 weeks (2), 8 weeks (1)

Day 6: none (0), 1 week (4), 2 weeks (28), 8 weeks (48)

Result 2: Duration of gluten free diet and IFNγ ELISpot responses on day 0 and day 6 of gluten challenge: tTG-gliadin (results expressed as IFNγ specific spots/million PPBMC)

Day 0: none (45), 1 week (62), 2 weeks (5), 8 weeks (5)

Day 6: none (0), 1 week (67), 2 weeks (40), 8 weeks (60)

Result 3: Duration of gluten free diet and IFNγ ELISpot responses on day 0 and day 6 of gluten challenge: A-gliadin 57-73 P65 (results expressed as IFNγ specific spots/million PPBMC)

Day 0: none (1), 1 week (2), 2 weeks (1), 8 weeks (1)

Day 6: none (0), 1 week (0), 2 weeks (0), 8 weeks (0)

Result 4: Duration of gluten free diet and IFNγ ELISpot responses on day 0 and day 6 of gluten challenge: PPD (results expressed as IFNγ specific spots/million PPBMC)

Day 0: none (90), 1 week (88), 2 weeks (210), 8 weeks (150)

Day 6: none (0), 1 week (100), 2 weeks (210), 8 weeks (100)

Result 5: Duration of gluten free diet and IFNγ ELISpot responses on day 0 and day 6 of gluten challenge: tTG (results expressed as IFNγ specific spots/million PPBMC)

Day 0: none (5), 1 week (4), 2 weeks (3), 8 weeks (2)

Day 6: none (0), 1 week (4), 2 weeks (1), 8 weeks (2)

Gluten challenge in HLA-DQ2 coeliac disease on long term gluten

Characterization of anti-gliadin T cell response was carried out in peripheral blood on day 6-8 after 3-day gluten challenge.

Result 1: PBMC Day 6 Long-term gluten free diet (preincubation with anti-HLA-DR and -DQ antibody) (expressed as % inhibition)

DR-: tTG-gliadin 100 mcg/ml (105), A-gliadin 57-73 QE65 50 mcg/ml (90), PPD 5 mcg/ml (30)

DQ-: tTG-gliadin 100 mcg/ml (5), A-gliadin 57-73 QE65 50 mcg/ml (22), PPD 5 mcg/ml (78).

Result 2: PBMC Day 6 Long-term gluten free diet (expressed as % CD8-depleted PBMC response)

B7 depletion: tTG-gliadin n=6 (7), A-gliadin 57-73 n=9 (6), PPD n=8 (62)

AE depletion: tTG-gliadin n=6 (120), A-gliadin 57-73 n=9 (80), PPD n=8 (110).

CD4 depletion: tTG-gliadin n=6 (10), A-gliadin 57-73 n=9 (9), PPD n=8 (10).

Therapeutic peptides include, but are not limited to

QLQPFPQPQLPYPQPQS(SEQ ID NO:10) (AG01)
QLQPFPQPQLPYPQPQP(SEQ ID NO:26) (AG02)
QLQPFPQPQLPYPQPQL(SEQ ID NO:51) (AG03)
QLQPFPQPQLPYLQPQP (SEQ ID NO:67) (AG04)
QLQPFPRPQLPYPQPQP (SEQ ID NO:68) (AG05)
QLQPFPQPQLPYSQPQP(SEQ ID NO:28) (AG06)
QLQPFLQPQLPYSQPQP(SEQ ID NO:69) (AG07)
QLQPFSQPQLPYSQPQP(SEQ ID NO:70) (AG08)
QLQPFPQPQLSYSQPQP(SEQ ID NO:71) (AG09)
PQLPYPQPQLPYPQPQP(SEQ ID NO:72) (AG10)
PQLPYPQPQLPYPQPQL(SEQ ID NO:73) (AG11)
PQPQPFLPQLPYPQPQS(SEQ ID NO:74) (AG12)
PQPQPFPPQLPYPQPQS(SEQ ID NO:75) (AG13)
PQPQPFPPQLPYPQYQP(SEQ ID NO:76) (AG14)
PQPQPFPPQLPYPQPPP(SEQ ID NO:77) (AG015)

Briefly after oral antigen challenge, specificities of peripheral blood T cells reflect those of intestinal T cell clones. In peripheral blood, epitopes of intestinal T cell clones are sub-optimal compared to A-gliadin 57-73 QE65, which is an optimal α-gliadin epitope.

Example 15

ELISpot assays were also carried out for mapping purposes as follows.

Fine-mapping the dominant DQ-8 associated epitope

Sequence / sfc	tTG-treated sequence / sfc
VPQLQPQNPSQQQPQEQV / 76(A)	RWPVPQLQPQNPSQQ / 60(L)
	WPVPQLQPQNPSQQQ / 90(M)

VPQLQPENPSQQQPQEQV / 3(B) PVPQLQPQNPSQQQP / 130(N)
VPQLQPRNPSQQQPQEQV / 76(C) VPQLQPQNPSQQQPQ / 140(O)
PQLQPQNPSQQQPQE / 59(P)
VPQLQPQNPSQEQPQEQV / 100(D) QLQPQNPSQQQPQEQ / 95(Q)
VPQLQPQNPSQRQPQEQV / 1(E) LQPQNPSQQQPQEQV / 30(R)
QPQNPSQQQPQEQVP / 4(S)
VPQLQPQNPSQQQPEEQV / 71(F)
VPQLQPQNPSQQQPREQV / 27(G) -----DQ8 Gliadin Epitope
GDA09 202Q / 6
VPQLQPQNPSQEQPEEQV / 81(H) GDA09 202E / 83
VPQLQPENPSQQQPEEQV / 2(I) GDA09 202Q+tTG / 17
VPQLQPENPSQEQPQEQV / 6(J) BI + tTG / 0
VPQLQPENPSQEQPEEQV / 5(K) BI / 0

A=SEQ ID NO:78
B=SEQ ID NO:79
C=SEQ ID NO:80
D=SEQ ID NO:81
E=SEQ ID NO:82
F=SEQ ID NO:83
G=SEQ ID NO:84
H=SEQ ID NO:85
I=SEQ ID NO:86
J=SEQ ID NO:87
K=SEQ ID NO:88
L=SEQ ID NO:89
M=SEQ ID NO:90
N=SEQ ID NO:91
O=SEQ ID NO:92

P=SEQ ID NO:93
Q=SEQ ID NO:94
R=SEQ ID NO:95
S=SEQ ID NO:96

Fine-mapping dominant epitope (2)

Pool 33 (deamidated) / sfc

A2b3 301 qqyp sgqg ffqp sqqn pqaq (SEQ ID NO:359)/ 2
A2b5 301 qqyp sgqg ffqp fqqn pqaq (SEQ ID NO:360)/ 1
A3a1 301 qqyp sgqg ffqp sqqn pqaq (SEQ ID NO:361)/ 0
A3b1 301 qqyp ssqv sfqp sqln pqaq (SEQ ID NO:362)/ 0
A3b2 301 qqyp ssqg sfqp sqqn pqaq (SEQ ID NO:363)/ 2
A4a 301 eqyp sgqv sfqs sqqn pqaq (SEQ ID NO:364)/ 28
A1b1 309 sfrp sqqn plaq gsvq pqql (SEQ ID NO:365)/ 2
A1a1 309 sfrp sqqn pqaq gsvq pqql (SEQ ID NO:366)/ 2

Example 16

Bioactivity of gliadin epitopes in IFNγ-ELISpot (25 mcg/ml, n=6) (expressed as % A-gliadin 57-73 QE65 response)

DQ2-AII: wild type (WT) (4), WT + tTG (52), Glu-substituted (52)
DQ2-AI: wild type (WT) (2), WT + tTG (22), Glu-substituted (28)
GDA09: wild type (WT) (1), WT + tTG (7), Glu-substituted (8)
A-G31-49: wild type (WT) (2), WT + tTG (3), Glu-substituted (0)

Dose response of A-Gliadin 57-73 QE65 (G01E) (n=8) (expressed as %G01E maximal response)

0.025 mcg/ml (1), 0.05 mcg/ml (8), 0.1 mcg/ml (10), 0.25 mcg/ml (22), 0.5 mcg/ml (38), 1 mcg/ml (43), 2.5 mcg/ml (52), 5 mcg/ml (70), 10 mcg/ml (81), 25 mcg/ml (95), 50 mcg/ml (90), 100 mcg/ml (85).

IFNγ ELISpot response to gliadin epitopes alone or mixed with A-gliadin 57-75 (G01E) (all 50 mcg/ml, tTG-gliadin 100 mcg/ml, PPD 5 mcg/ml; n=9) (expressed as % G01E response)

Alone: DQ2-A1 (20), DQ2-A2 (55), Omega G1 (50), tTG Gliadin (80), PPD (220), DQ2 binder (0)

G01E+: DQ2-A1 (90), DQ2-A2 (95), Omega G1 (100), tTG Gliadin (120), PPD (280), DQ2 binder (80)

Effect of alanine and lysine substitution of A-gliadin 57-73 QE65 on IFNγ ELISpot responses in individual coeliac subjects (n=8)

Epitope sequence: QLQPFPQPELPYPQPQS(SEQ ID NO:2)

Alanine substitution at positions 57-59 and 72-73 showed little to no decrease in % A-gliadin 57-73 QE65 response. Alanine substitution at positions 60-62 and 68-71 showed moderate decrease in % A-gliadin 57-73 QE65 response. Alanine substitution at positions 63-67 showed most decrease in % A-gliadin 57-73 QE65 response.

Effect of lysine substitution of A-gliadin 57-73 QE65 on IFNγ ELISpot responses in individual coeliac subjects (n=8);

Epitope sequence: QLQPFPQPELPYPQPQS(SEQ ID NO:2)

Lysine substitution at positions 57-59 and 71-73 showed little to no decrease in % A-gliadin 57-73 QE65 response. Lysine substitution at positions 60-61 and 69-70 showed moderate decrease in % A-gliadin 57-73 QE65 response. Lysine substitution at positions 62-68 showed most decrease in % A-gliadin 57-73 QE65 response.

Example 17

Table 24 shows the results of analyses examining the 652 peptides with several patients challenged with wheat or rye.

References

1. Molberg O, et al. Nature Med. 4, 713-717 (1998).

2. Quarsten H, et al. Eur. J. Immunol. 29, 2506-2514 (1999).

3. Greenberg CS et al. FASEB 5, 3071-3077 (1991).

4. Mantzaris G, Jewell D. Scand. J. Gastroenterol. 26, 392-398 (1991).

5. Mauri L, et al. Scand. J. Gastroenterol. 31, 247-253 (1996).

6. Bunce M, et al. Tissue Antigens 46, 355-367 (1995).

7. Olerup O, et al. Tissue antigens 41, 119-134 (1993).

8. Mullighan CG, et al. Tissue-Antigens. 50, 688-92 (1997).

9. Plebanski M et al. Eur. J. Immunol. 28, 4345-4355 (1998).

10. Anderson DO, Greene FC. The alpha-gliadin gene family. II. DNA and protein sequence variation, subfamily structure, and origins of pseudogenes. Theor Appl Genet (1997) 95:59-65.

11. Arentz-Hansen H, Korner R, Molberg O, Quarsten H, Van der Wal Y, Kooy YMC, Lundin KEA, Koning F, Roepstorff P, Sollid LM, McAdam SN. The intestinal T cell response to alpha–gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. J Exp Med. 2000; 191:603-12.

12. Vader LW, de Ru A, van der Wal, Kooy YMC, Benckhuijsen W, Mearin ML, Drijfhout JW, van Veelen P, Koning F. Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. J Exp Med 2002; 195:643-649.

13. van der Wal Y, Kooy Y, van Veelan P, Pena S, Mearin L, Papadopoulos G, Koning F. Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. J Immunol. 1998; 161:1585-8.

14. van der Wal Y, Kooy Y, van Veelan P, Pena S, Mearin L, Molberg O, Lundin KEA, Sollid L, Mutis T, Benckhuijsen WE, Drijfhout JW, Koning F. Proc Natl Acad Sci USA 1998; 95:10050-10054.

15. Vader W, Kooy Y, Van Veelen P et al. The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. Gastroenterology 2002, 122:1729-37

16. Arentz-Hansen H, McAdam SN, Molberg O, et al. Celiac lesion T cells recognize epitopes that cluster in regions of gliadin rich in proline residues. Gastroenterology 2002, 123:803-809.

Each of the PCT publications, U.S. patents, other patents, journal references, and any other publications cited or referred to herein is incorporated herein by reference in their entirety.

Table 1. A-Gliadin protein sequence (based on amino acid sequencing) (SEQ ID NO:3)

```
VRVPVPQLQP QNPSQQQPQE QVPLVQQQQF PGQQQQFPPQ QPYPQPQPFP SQQPYLQLQP FPQPQLPYPQ
1          11         21         31         41         51         61
PQSFPPQQPY PQPQPQYSQP QQPISQQQAQ QQQQQQQQQQ QQQILQQILQ QQLIPCMDVV LQQHNIAHAR
71         81         91         101        111        121        131
SQVLQQSTYQ LLQELCCQHL WQIPEQSQCQ AIHNVVHAII LHQQQKQQQQ PSSQVSFQQP LQQYPLGQGS
141        151        161        171        181        191        201
FRPSQQNPQA QGSVQPQQLP QFEEIRNLAL QTLPAMCNVY IAPYCTIAPF GIFGTN
211        221        231        241        251        261
```

Table 2. Coeliac disease subjects studied

	Age Sex	Gluten free diet	HLA-DQ2	Bread challenge	Symptoms with bread
1	64 f	14 yr	Homozygote	3 days	Abdominal pain, lethargy, mouth ulcers, diarrhoea
2	57 m	1 yr	Heterozygote	10 days	Lethargy, nausea
3	35 f	7 yr	Heterozygote	3 days	Nausea
4	36 m	6 wk	Homozygote	3 days	Abdominal pain, mouth ulcers, diarrhoea
5	26 m	19 yr	Heterozygote	3 days	None
6	58 m	35 yr	Heterozygote	3 days	None
7	55 m	1 yr	Heterozygote	3 days	Diarrhoea
8	48 f	15 yr	Homozygote	3 days	Abdominal pain, diarrhoea

Aminoacid at position	Range	Mean
Glutamate	(100)	100%
Asparagine	(50-84)	70%
Aspartate	(50-94)	65%
Alanine	(44-76)	64%
Cysteine	(45-83)	62%
Serine	(45-75)	62%
Valine	(24-79)	56%
Threonine	(46-66)	55%
Glycine	(34-47)	40%
Leucine	(8-46)	33%
Glutamine	(16-21)	19%
Isoleucine	(3-25)	14%
Methionine	(3-32)	14%
Phenylaleucine	(0-33)	12%
Histidine	(0-13)	8%
Tyrosine	(0-17).	8%
Tryptophan	(0-17)	8%
Lysine	(o-11)	4%
Proline	(0-4)	2%
Arginine	(0-2)	1%

Table 3

pi response		Peptics sequence	Corresponding residues in gliadin protein sequences (Accession no)	
13).		QLQPSPQPQLPYPQPQ (SEQ ID NO:10)	57-73	α-Gliadin (T.aestivum) Q41545
	100(100)	QLQPFPQPELPVPQPQS (SEQ ID NO:62)	57-73	α-Gliadin (T.aestivum) Q41545
7)	53(44-67)	QLQPFPQPQLPYSQPQP (SEQ ID NO:28)	77-93	α/β-Gliadin precursor (Tricetum.aestivum) P02863
			76-92	α-Gliadin (T.aestivum) Q41528
			77-93	α-Gliadin storage protein (T.aestivum) Q41531
			, 57-73	α-Gliadin mature peptide (T.aestivum) Q41533
			77-93	α-Gliadin precursor (T. spelta) Q9ZP09
-20)	83 (61-113)	QLQPFPQPQLPYPQPQP' (SEQ ID NO:26)	77-93	α/β-Gliadin A-II precursor (T.aestivum) P0472
-337)	83(74-97)	QLQPFPQPQLFVFQPQL (SEQ ID NO:51)	77-93	α/β-Gliadin A-IV precursor (T.aestivum)P04724
			77-93	α/β-Gliadin MM1 precursor (T.aestivum)P18573
	1O9(41-152))	PQLPYPQPQLPYPQPQP (SEQ ID NO:72)	84-100	α/β-Gliadin A-IV precursor (T.aestivum) P04724
		PQLPYPQPQLPYPQPQL (SEQ ID NO:73)	84-100	α/β-Gliadin MM1 precursor (T.aestivum)P18573
	3(0-7)	QLQPFLQPQLPYSQPQP (SEQ ID NO:69)	77-93	α/β-Gliadin A-I precursor (T.aestivum) P04721
			77-93	α-Gliadin (T.aestivum) Q41509
	2(0-7)	QLQPFSQPQLPYSQPQP (SEQ ID NO:79)	77-93	α-Gliadin storage protein (T.aestivum) Q41530
		PQPQPFPQLPYPQTQP (SEQ ID NO:97)	77-93	α/β-Gliadin A-III precursor (T.aestivum) P04723
-40)	24(11-43)	PQPQPFPPQLPYPQPQS (SEQ ID NO:75)	82-98	α/β-Gliadin A-V precursor (T.aestivum) P04725
-30)	19(11-33)	PQPQPFPPQLPYPQPPP (SEQ ID NO:77	82-98 82-98	α/β-Gliadin clone PW1215 precursor (T.aestivum) P04726 α/β-Gliadin (T.urartu) Q41632
-30)	21(11-33)	PQPQPFLPQLPYPQPQS (SEQ ID NO:74)	79-95	α/β-Gliadin clone PW8142 precursor (T.aestivum) P04726
			79-95	α-Gliadin (T.aestivum) Q41529
			79-95	α/β-Gliadin precursor (T.aestivum) Q41546

Table 4

Table 5. Table 5 T cell epitopes described in coeliac disease

Source	Restriction	Frequency	Sequence*
Gamma-gliadin	DQ2	3/NS (iTCC)	QQLPQPEQPQQSFPEQERPF(SEQ
Alpha-gliadin	DQ2	12/17 (iTCL)	QLQPFPQPELPY(SEQ ID NO:13)
Alpha-eliadin	DQ2	11/17 (iTCL)	PQPELPYPQPELPY(SEQ ID
Alpha-gliadin	DQ2	1/23 (bTCC)	LQQQQPFPQQPYPQPQPF(SEQ
Alpha-gliadin	DQ8	3/NS (iTCC)	QQYPSGEGSFQPSQENPQ(SEQ ID
Glutenin	DQ8	1/1 (iTCC)	GQQGYYPTSPQQSGQ(SEQ ID
Alpha-gliadin	DQ2	11/12 in vivo	QLQPFPQPELPYPQPQS(SEQ ID

NS not stilted in original publication, iTCC intestinal T cell clone, iTCL intestinal polyclonal T cell line, bTCC peripheral bl T cell clone. All peptides are the products of transglutaminase modifying wild type gluten peptides except the fourth and sixth peptides

Table 6. Relative bioactivity of gliadin T cell epitopes in coeliac PBMC after gluten challenge

. ELISpot response as % A-gliadin 57-73 QE65 (all 25mcg/ml)

Sequence	Wild type	Wildtype+ITG	E-substituted
QQLPQPEQPQQSFPEQERPF(SEQ ID NO:48)	9(3)	18(7)	10(5)
QLQPFPQPELPY(SEQ ID NO:13)	6(2)	19(1)	8(3)
PQPELPYPQPELPY(SEQ ID NO:47)	13(6)	53(8)	48(9)
QQYPSGEGSFQPSQENPQ(SEQ ID NO:14)	10(3)	9(3)	14(8)
QLQPFPQPELPYPQPQS(SEQ ID NO:2)	18(7)	87(7)	100
PQLPYPQPELPYPQPQP(SEQ ID NO:101)	14(4)	80 (17)	69(20)

Sequence refers that of transglutaminase (tTG) modified peptide and the t cell epitope. Wild type is the unmodified gliadin peptide. Data from 4 subjects. Blankwas5 (1)%.

Table 7. Polymorphisms of A-gliadin 57-73

A. Sequences derived from Nordic autumn wheat strain Mjoelner

Alpha-gliadin protein (single letter code refers to Fig. 14 peptides)	Polymorphism
Q41545 A-gliadin (from sequenced protein) 57-73 (A)	QLQPFPQPQLPYPQPQS
Gli alpha 1,6: (EMBL: AJ133605 & AJ133602 58-74) (J)	(SEQ ID NO:10)
Gli alpha 3,4,5: (EMBL: AJ133606, AJ133607, AJ133608 57-73) (I)	QPQPFPPPQLPYPQTQP
Gli alpha 7: (EMBL: AJ133604 57-73) (E)	(SEQ ID NO:105)
Gli alpha 8, 9, 11: (EMBL:) (F)	QLQPFPQPQLSYSQPQP
Gli alpha 10: (EMBL: AJ133610 57-73) (D)	(SEQ ID NO:71)
	QLQPFPRPQLPYPQPQP
	(SEQ ID NO:68)
	QLQPFPQPQLPYSQPQP
	(SEQ ID NO:28)
	QLQPFPQPQLPYLQPQS
	(SEQ ID NO:104)

B. SWISSPROT and TREMBL scan (10.12.99) for gliadins containing the sequence: XXXXXXXPQLPYXXXXX

Wheat (Triticum aestivum unless stated) gliadin accession number	Polymorphism
Q41545 A-gliadin (from sequenced protein) 57-73 (A)	QLQPFPQPQLPYPQPQS(SEQ ID NO:10)
SWISSPROT:	
GDA0_WHEAT P02863 77-93 (F)	QLQPFPQPQLPYSQPQP(SEQ ID NO:28)
GDA1_WHEAT P04721 77-93 (G)	QLQPFLQPQLPYSQPQP(SEQ ID NO:69)
GDA2_WHEAT P04722 77-93 (B)	QLQPFPQPQLPYPQPQP(SEQ ID NO:26)
GDA3_WHEAT P04723 77-93 (O)	PQPQPFPPQLPYPQTQP(SEQ ID NO:97)
GDA4_WHEAT P04724 77-93 (C)	QLQPFPQPQLPYPQPQL(SEQ ID NO:51)
GDA4_WHEAT P04724 84-100 (K)	PQLPYPQPQLPYPQPQP(SEQ ID NO:72)
GDA5_WHEAT P04725 82-98 (N)	PQPQPFPPQLPYPQPQS(SEQ ID NO:75)
GDA6_WHEAT P04726 82-98 (P)	PQPQPFPPQLPYPQPPP(SEQ ID NO:77)
GDA7_WHEAT P04727 79-95 (M)	PQPQPFLPQLPYPQPQS(SEQ ID NO:74)
GDA9_WHEAT P18573 77-93 (C)	QLQPFPQPQLPYPQPQL(SEQ ID NO:51)
GDA9_WHEAT P18573 84-100 (L)	PQLPYPQPQLPYPQPQL(SEQ ID NO:73)
GDA9_WHEAT P18573 91-107 (K)	PQLPYPQPQLPYPQPQP(SEQ ID NO:72)
TREMBL	
Q41509 ALPHA-GLIADIN 77-93 (G)	QLQPFLQPQLPYSQPQP(SEQ ID NO:69)
Q41528 ALPHA-GLIADIN 76-92 (F)	QLQPFPQPQLPYSQPQP(SEQ ID NO:28)
Q41529 ALPHA-GLIADIN 79-95 (M)	PQPQPFLPQLPYPQPQS(SEQ ID NO:74)
Q41530 ALPHA-GLIADIN 77-93 (H)	QLQPFSQPQLPYSQPQP(SEQ ID NO:70)
Q41531 ALPHA-GLIADIN 77-93 (F)	QLQPFPQPQLPYSQPQP(SEQ ID NO:28)
Q41533 ALPHA-GLIADIN 57-73 (F)	QLQPFPQPQLPYSQPQP(SEQ ID NO:28)
Q41546 ALPHA/BETA-GLIADIN 79-95 (M)	PQPQPFLPQLPYPQPQS(SEQ ID NO:74)
Q41632 ALPHA/BETA-TYPE GLIADIN. Triticum urartu 82-98 (P)	PQPQPFPPQLPYPQPPP(SEQ ID NO:77)
Q9ZP09 ALPHA-GLIADIN Triticum spelta 77-93 (F)	QLQPFPQPQLPYSQPQP(SEQ ID NO:28)

Table 8. Bioactivity of substituted variants of A-gliadin 57-73 QE65 (Subst) compared to unmodified A-gliadin 57-73 QE65 (G) (mean 100%, 95% CI 97-104) and blank (no peptide, bl) (mean 7.1%, 95% CI: 5.7-8.5)

Subst	%	P vs G	Subst	%	P vs G	Subst	%	P vs G	Subst	%	P vs G	P vs bl
Super-agonists			F62	71	0.001	H62	47	<0.0001	N66	24	<0.0001	
Y61	129	<0.0001	V63	70	<0.0001	G69	47	<0.0001	R64	24	<0.0001	
Y70	129	0.0006	S69	70	<0.0001	N63	47	<0.0001	K63	23	<0.0001	
Agonists			H63	70	<0.0001	H68	47	<0.0001	V65	23	<0.0001	
W70	119	0.017	F63	70	0.008	M68	46	<0.0001	H66	23	<0.0001	
K57	118	0.02	P70	69	<0.0001	D68	46	<0.0001	H67	22	<0.0001	
Y59	117	0.04	T62	69	<0.0001	V69	46	<0.0001	L64	22	<0.0001	
A57	116	0.046	L61	69	<0.0001	G63	45	<0.0001	S66	22	<0.0001	
S70	116	0.045	S61	69	<0.0001	V64	45	<0.0001	F67	21	<0.0001	
K58	114	0.08	T61	69	<0.0001	E61	45	<0.0001	W66	21	<0.0001	
W59	110	0.21	T63	69	<0.0001	A69	43	<0.0001	G64	21	<0.0001	
A73	109	0.24	M66	68	<0.0001	R62	42	<0.0001	G65	21	<0.0001	
I59	108	0.37	T69	67	<0.0001	G68	42	<0.0001	D64	21	<0.0001	
G59	108	0.34	K60	66	<0.0001	A64	42	<0.0001	I65	21	<0.0001	
A58	108	0.35	S62	66	<0.0001	C65	42	<0.0001	M64	20	<0.0001	<0.0001
W60	105	0.62	M61	66	<0.0001	N67	41	<0.0001	G67	19	<0.0001	<0.0001
A59	104	0.61	P61	65	<0.0001	W63	41	<0.0001	T65	19	<0.0001	0.003
K72	104	0.65	M62	64	<0.0001	F69	41	<0.0001	A66	19	<0.0001	<0.0001
S59	103	0.76	Q61	64	<0.0001	N68	40	<0.0001	I64	19	<0.0001	0.0003
K73	102	0.8	G61	64	<0.0001	V66	40	<0.0001	R63	19	<0.0001	<0.0001
A70	102	0.81	A63	64	<0.0001	H69	40	<0.0001	W67	19	<0.0001	<0.0001
Y60	101	0.96	L62	60	<0.0001	M69	40	<0.0001	K68	18	<0.0001	<0.0001
A72	100	0.94	I68	60	<0.0001	R69	40	<0.0001	H64	18	<0.0001	<0.0001
S63	98	0.67	S67	59	<0.0001	W69	40	<0.0001	W64	18	<0.0001	0.0001
K59	96	0.46	N61	59	<0.0001	Q69	39	<0.0001	Q65	18	<0.0001	0.0002
I60	96	0.5	I69	59	<0.0001	L67	38	<0.0001	F64	16	<0.0001	0.0008
G70	95	0.41	V61	58	<0.0001	K69	38	<0.0001	L65	16	<0.0001	0.0022
D65	95	0.44	D61	58	<0.0001	K62	38	<0.0001	N64	16	<0.0001	<0.0001
E70	93	0.27	E60	57	<0.0001	E67	37	<0.0001	F65	16	<0.0001	0.12
I63	92	0.19	A61	57	<0.0001	L69	37	<0.0001	Q67	15	<0.0001	0.0012
S60	92	0.23	Q62	56	<0.0001	S64	36	<0.0001	M65	14	<0.0001	0.015
P59	88	0.08	F68	56	<0.0001	G62	36	<0.0001	D66	14	<0.0001	0.013
M63	87	0.03	N65	56	<0.0001	E69	36	<0.0001	R67	14	<0.0001	0.002
K71	85	0.047	A62	56	<0.0001	E68	36	<0.0001	Non-agonists			
V62	84	0.04	A68	53	<0.0001	V67	35	<0.0001	P63	13	<0.0001	0.012
I70	84	0.04	P66	53	<0.0001	D62	35	<0.0001	E64	12	<0.0001	0.053
I61	83	0.01	R61	53	<0.0001	R68	34	<0.0001	W65	11	<0.0001	0.24
V68	82	0.0045	S68	53	<0.0001	Q66	34	<0.0001	Q64	11	<0.0001	0.15
E59	81	0.01	Y63	52	<0.0001	A67	33	<0.0001	G66	11	<0.0001	0.07
Partial agonists			N69	51	<0.0001	N62	32	<0.0001	R65	11	<0.0001	0.26
W61	79	0.002	E63	51	<0.0001	F66	31	<0.0001	Y67	10	<0.0001	0.13
A60	78	0.002	T64	51	<0.0001	E62	31	<0.0001	E66	10	<0.0001	0.17
Y62	78	0.006	T67	51	<0.0001	D69	31	<0.0001	K66	10	<0.0001	0.21
G60	77	0.003	Y69	50	<0.0001	D67	30	<0.0001	R66	10	<0.0001	0.23
A71	77	0.003	D63	50	<0.0001	M67	29	<0.0001	K67	10	<0.0001	0.11
W62	76	0.0009	A65	49	<0.0001	Y66	28	<0.0001	P65	8	<0.0001	0.57
Q60	76	0.001	K61	49	<0.0001	I67	28	<0.0001	K64	8	<0.0001	0.82
L63	74	0.0002	I66	49	<0.0001	H65	26	<0.0001	K65	8	<0.0001	0.63
I62	74	0.0005	T68	48	<0.0001	P68	26	<0.0001	Y65	7	<0.0001	0.9
K70	74	0.001	S65	48	<0.0001	Y64	25	<0.0001				
H61	72	<0.0001	L68	48	<0.0001	EK65	25	<0.0001				
W68	72	<0.0001	Q68	48	<0.0001	T66	25	<0.0001				

Table 9. Antagonism of A-gliadin 57-73 QE65 interferon gamma ELISPOT response by substituted variants of A-gliadin 57-73 QE65 (Subst) (P is significance level in unpaired t-test). Agonist activity (% agonist) of peptides compared to A-gliadin 57-73 QE65 is also shown.

Subst	% Inhibit.	P	% agonist.	Subst	% Inhibit.	P	% agonist.
Antagonists			65R	13	0.18	11	
65T	28	0.004	19	65M	13	0.16	14
67M	27	0.0052	29	68P	13	0.16	26
64W	26	0.007	18	63R	13	0.19	19
67W	25	0.0088	19	66G	12	0.19	11
Potential antagonists				65Q	12	0.2	18
67I	24	0.013	10	65Y	12	0.22	7
67Y	24	0.013	21	66S	12	0.22	22
64G	21	0.03	21	67F	11	0.25	21
64D	21	0.029	16	66R	10	0.29	10
65L	20	0.046	26	67K	10	0.29	10
66N	20	0.037	24	64F	10	0.29	16
65H	20	0.038	16	65F	9	0.41	16
64N	19	0.05	16	63P	8	0.42	13
64Y	19	0.06	25	65EK	8	0.39	25
66Y	19	0.048	28	64Q	7	0.49	11
64E	19	0.049	12	64I	5	0.6	21
67A	18	0.058	30	68K	5	0.56	19
67H	18	0.052	22	67Q	5	0.61	18
Non-antagonists			65G	5	0.62	15	
65V	17	0.07	23	64M	4	0.7	20
65I	17	0.086	21	66H	4	0.66	23
66T	17	0.069	25	66 E	3	0.76	10
65W	15	0.11	11	66D	1	0.9	14
67R	15	0.13	14	63K	1	0.88	23
65P	15	0.13	8	64H	1	0.93	18
65K	15	0.11	8	66K	0	0.98	10
66W	15	0.12	21	64K	-2	0.88	8
67G	14	0.14	19	64L	-11	0.26	22
66A	14	0.14	19				

Table 10. Inhibition of A-gliadin 57-73 QE65 interferon gamma ELISPOT response by peptides known to bind HLA-DQ2 (P is significance level in unpaired t-test).

Peptide	% Inhibit.	P
TP	31	<0.0001
HLA1a	0	0.95

Table 11. Antagonism of A-gliadin 57-73 QE65 interferon gamma ELISpot response by naturally occurring polymorphisms of A-gliadin 57-73 QE65 (P is significance level in unpaired t-test).

A-gliadin 57-73 QE65 polymorphism		% Inhibit.	P
P04725 82-98 QE90 (SEQ ID NO:17)	PQPQPFPPELPYPQPQS	19	0.009
Q41509 77-93 QE85	QLQPFLQPELPYSQPQP	11	0.15
Gli ∀ 1,6 58-74 QE66	QPQPFPPPELPYPQTQP	11	0.11
P04723 77-93 QE85	PQPQPFPPELPYPQTQP	10	0.14
Gli ∀ 3-5 57-73 QE65	QLQPFPQPELSYSQPQP	7	0.34
P02863 77-93 QE85	QLQPFPQPELPYSQPQP	6	0.35
Q41509 77-93 QE85 (SEQ ID NO:29)	QLQPFLQPELPYSQPQP	6	0.41
P04727 79-95 QE65	PQPQPFLPELPYPQPQS	6	0.39
P04726 82-98 QE90	PQPQPFPPELPYPQPPP	5	0.43

Table 12. Prolamin homologues of A-gliadin 57-73 (excluding alpha/beta-gliadins)

Prolamin	Accession number	Sequence	% Bioactivity*
Wheat: α-gliadin	A-gliadin (57-73)	**QLQPFPQPQLPYPQPQS**(SEQ ID NO:16)	100 (0)
Wheat: ω-gliadin	AAG17702 (141-157)	PQ……F……QSE	32 (6.4)
Barley: C-hordein	Q40055 (166-182)	…QPFPL……F……Q	2.3 (2.0)
Wheat γ-gliadin	P21292 (96-112)	…QTFPQ……F……QPQ	2.1 (4.2)
Rye: secalin	Q43639 (335-351)	…QPSPQ……F……Q	1.6 (1.4)
Barley: γ-hordein	P80198 (52-68)	…QPFPQ……HQHQFP	-1.0 (1.8)
Wheat: LMW glutenin	P16315 (67-83)	LQ…QPIL……FS…Q…Q	-0.9 (1.0)
Wheat: HMW glutenin	P08489 (718-734)	HGYYPTS……SGQGQRP	6.4 (4.0)
Wheat γ-gliadin	P04730 (120-136)	…QCCQQL……I…QQSRYQ	0.7 (0.9)
Wheat: LMW glutenin	P10386 (183-199)	…QCCQQL……I…QQSRYE	-0.7 (0.5)
Wheat: LMW glutenin	O49958 (214-230)	…QCCRQL……I…EQSRYD	-1.1 (0.3)
Barley: B1-hordein	P06470 (176-192)	…QCCQQL……I…EQFRHE	1.8 (1.4)
Barley: B-hordein	Q40026 (176-192)	…QCCQQL……ISEQFRHE	0.5 (0.9)

*Bioactivity is expressed as 100x(spot forming cells with peptide 25mcg/ml plus tTG 8mcg/ml minus blank)/(spot forming cells with A-gliadin 57-73 25mcg/ml plus tTG 8mcg/ml minus blank) (mean (SEM), n=5). Peptides were preincubated with tTG for 2h 37°C. Note, Q is deamidated in A-gliadin 57-73 by tTG.

Table 13. Clinical details of coeliac subjects.

	HLA-DQ	HLA-DQA1 alleles	HLA-DQB1 alleles	Duodenal histology	Gluten free	EMA on gluten (on GFD)
C01	2, 6	102/6, 501	201, 602	SVA	1 yr	+ (-)
C02	2, 2	501	201	SVA	1 yr	+ (-)
C03	2, 5	101/4/5, 501	201, 501	PVA	1 yr	+ (-)
C04	2,5	101/4-5, 501	201, 501	SVA	7 yr	+ (-)
C05	2, 2	201, 501	201, 202	SVA	4 mo	+ (ND)
C06	2, 2	201, 501	201, 202	SVA	2 yr	+ (-)
C07	2, 8	301-3, 501	201, 302	SVA	1 yr	+ (-)
C08	2, 8	301-3, 501	201, 302/8	SVA	11 yr	ND (-)
C09	2, 8	301-3, 501	201, 302	SVA	29 yr	+ (-)
C10	2, 8	201, 301-3	202, 302	IEL	1 yr	+ (-)
C11	6,8	102/6, 301-3	602/15, 302/8	IEL	9 mo	- (ND)
C12	8,7	301-3, 505	302, 301/9-10	SVA	2 yr	- (-)
C13	8, 8	301	302	SVA	1 yr	+ (+)

SVA subtotal villous atrophy, PVA partial villous atrophy, IEL increased intra-epithelial atrophy, GFD gluten-free diet, ND not done.

Table 14. HLA-DQ2+ Coeliac (C01-6) and healthy control (H01-10) IFNγ ELISpot responses to control peptides (20 µg/ml) and gliadin (500 µg/ml) before and after gluten challenge (sfc/million PBMC minus response to PBS alone)

Peptide	Healthy Day 0	Healthy Day 6	Coeliac Day 0	Coeliac Day 6
P04722 77-93	0 (-4 to 17)	0 (-5 to 9)	-2 (-3 to 0)	**27 (0-100)***
P04722 77-93 + tTG	0 (-5 to 4)	0 (-9 to 3)	0 (-4 to 11)	**141 (8 to 290)****
P04722 77-93 QE85	0 (-5 to 5)	0 (-3 to 4)	0 (-6 to 14)	**133 (10 to 297)***
P02863 77-93	0 (-4 to 13)	2 (-3 to 5)	-2 (-3 to 2)	**8 (-2 to 42)****
P02863 77-93 + tTG	-1 (-5 to 4)	-1 (-4 to 11)	1 (-4 to 6)	**65 (8-164)****
P02863 77-93 QE85	0 (-4 to 13)	0 (-4 to 14)	-1 (-4 to 6)	**42 (-2 to 176)***
Gliadin chymotrypsin	2 (-5 to 20)	**18 (0 to 185)***	20 (11 to 145)	92 (50 to 154)
Gliadin chymotrypsin + tTG	0 (-1 to 28)	**16 (-9 to 171)***	55 (29 to 248)	**269 (206 to 384)****
Chymotrypsin	0 (-4 to 5)	1 (-4 to 11)	-2 (-5 to 5)	1 (-4 to 8)
Chymotrypsin + tTG	0 (-5 to 8)	6 (0 to 29)	-2 (-3 to 11)	**2 (-3 to 18)***
Gliadin pepsin	4 (-4 to 28)	**29 (0 to 189)*****	44 (10 to 221)	**176 (54 to 265)****
Gliadin pepsin + tTG	2 (-3 to 80)	**27 (-4 to 241)*****	61 (8 to 172)	**280 (207 to 406)****
Pepsin	0 (-4 to 10)	0 (-3 to 12)	0 (-2 to 3)	2 (-2 to 8)
Pepsin + tTG	0 (-3 to 8)	0 (-5 to 9)	1 (-6 to 3)	0 (-3 to 14)
PBS alone	4 (0 to 6)	2 (0 to 6)	4 (1 to 12)	4 (0 to 4)
PBS + tTG	3 (0 to 8)	3 (0 to 11)	4 (2 to 10)	4 (2 to 11)

Day 6 vs. Day 0: *P<0.05 **P,0.02, ***P<0.01 by one-tailed Wilcoxon Matched-Pairs Signed-Ranks test

Table 15. Effect of deamidation by tTG to gliadin (0.5 mg/ml) and A-gliadin 57-73 homologues on IFNγ ELISpot responses in HLA-DQ2+ coeliac (C01-6) and healthy control subjects (H01-10) (median ratio tTG:no tTG pretreatment, range)

Peptide	Healthy Day 6	Coeliac Day 0	Coeliac Day 6
Gliadin chymotrypsin	0.94 (0.4-9.0)	2.1 (0.8-6.8)*	3.2 (1.8 -4.2)**
Gliadin pepsin	1.4 (0.5-1.4)	1.4 (0.8-4.0)*	1.9 (1.1-4.4)**
P04722 77-93 Q85			6.5 (2.3-12)**
P04722 77-93 E85			0.7 (0.6-1.1)
P02863 77-93 Q85			7.5 (3.9-19.9)**
P02863 77-93 E85			1.0 (0.8-1.2)

TTG>no tTG: *P<0.05 **P,0.02, ***P<0.01 by one-tailed Wilcoxon Matched-Pairs Signed-Ranks test

Table 16. Healthy subjects: IFNγ ELISpot Responses (>10 sfc/million PBMC and >4 x buffer only) to tTG-treated gliadin peptide Pools on Day 6 of gluten challenge (sfc/million PBMC) (*italic*: response also present on Day 0):

Group 1 – HLA-DQ2 (DQA1*0501-5, DQB1*0201)

Group 2 – HLA-DQ8 (DQA1*0301, DQB1*0302) and absent or "incomplete" DQ2 (only DQA1*0501-5 or DQB1*0201)

	Group 1										Group 2
Subject	H01	H02	H03	H04	H05	H06	H07	H08	H09	H10	H11
HLA-DQ	2, 6	2, 7	2, 8	2, 5	2, 6	2, 6	2, 6	2, 7	2, 5	2, 5	8, 8
Pool 1	.	.	.	.	.	.	.	.	.	.	.
2	.	.	.	.	.	.	.	.	.	.	.
3	.	.	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	.	.	.	13	.	.
5	.	17	.	.	.	.	.	.	*24*	.	.
6	.	.	.	.	.	.	.	.	*31*	.	.
7	.	.	.	.	.	.	.	.	.	.	.
8	.	.	.	.	.	.	.	.	.	.	.
9	.	.	.	.	.	.	.	.	.	.	.
10	.	.	.	.	.	.	.	.	.	.	.
11	.	.	.	.	.	.	.	.	.	.	.
12	.	.	.	.	.	.	.	.	.	.	.
13	.	.	.	.	.	.	.	.	.	.	.
14	.	.	.	.	.	.	.	.	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.
16	.	.	.	.	.	.	.	.	.	.	.
17	.	.	.	.	.	.	.	.	.	.	.
18	.	.	.	.	.	.	20	.	.	.	.
19	.	.	.	.	.	.	.	.	.	.	.
20	.	11	.	.	.	.	.	.	.	.	.
21	.	11	.	.	.	.	.	.	27	.	.
22	.	.	.	.	.	.	.	.	.	.	.
23	.	*43*	.	.	.	.	.	.	.	.	.
24	.	.	.	.	.	.	.	.	.	.	.
25	.	11	.	.	.	.	.	.	.	.	.
26	.	.	.	.	.	.	.	.	.	.	.
27	.	.	.	.	.	.	.	.	.	.	.
28	.	.	.	.	.	.	.	.	.	.	.
29	.	.	.	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	23	.	.	.
31	.	.	.	.	.	.	.	.	.	.	.
32	.	.	.	.	.	.	.	.	.	.	.
33	.	20	.	.	.	.	.	.	.	.	.
34	.	.	.	.	.	.	.	.	.	.	.
35	.	11	.	.	.	.	.	.	.	.	.
36	.	.	.	.	.	.	.	.	.	.	.
37	.	.	.	.	.	.	.	*18*	.	.	.
38	14	.	.	.	.	.	.	12	.	.	.
39	.	.	.	.	.	.	.	11	.	.	.
40	.	14	.	.	.	.	.	17	.	.	.

41	.	.	.	.	.	.	.	.	.	.	.	.
42	.	.	.	.	.	.	.	.	.	.	.	.
43	.	.	.	.	.	.	.	.	.	11	.	.
44	.	14	.	.	.	.	.	.	.	.	.	.
45	.	11	.	.	.	.	.	.	.	.	.	.
46	.	.	.	.	.	.	.	.	.	.	.	.
47	.	.	.	.	.	.	.	.	.	.	.	.
48	.	.	.	.	.	.	.	.	.	.	.	.
49	.	.	.	.	.	.	.	.	.	.	.	.
50	.	14	.	.	12	.	.	***22***	.	***14***	.	
51	.	.	.	.	.	.	.	.	.	.	.	.
52	.	14	.	.	.	.	.	.	.	.	.	.
53	.	26	.	.	.	.	.	.	.	.	.	.
54	.	.	.	.	.	.	.	12	.	.	.	.
55	.	.	.	.	.	.	.	.	.	.	.	.
56	.	.	.	.	.	.	.	.	.	.	.	.
57	.	23	.	.	.	.	12	.	.	.	.	.
58	.	14	.	.	.	.	.	.	.	.	.	.
59	.	.	.	.	.	.	.	.	.	.	.	.
60	.	.	.	.	.	.	.	.	.	.	.	.
61	.	23	.	.	.	.	.	***11***	***11***	.	.	.
62	.	.	.	.	.	.	.	.	.	.	.	.
63	.	.	.	.	.	.	.	.	.	.	.	.
64	.	20	.	.	.	.	.	.	.	.	.	.
65	.	.	.	.	.	.	.	.	.	.	.	.
66	.	14	.	.	.	.	.	.	.	.	.	.
67	.	11	.	.	.	.	.	.	.	.	.	.
68	.	20	.	.	.	.	.	***20***	.	.	.	.
69	.	20	.	.	.	.	.	.	.	.	.	.
70	.	.	.	.	.	.	.	.	.	.	.	.
71	.	.	.	.	.	.	.	.	.	.	.	16
72	.	11	.	.	.	.	.	.	.	.	.	.
73	.	14	.	.	.	.	.	.	.	.	.	.
74	.	.	.	.	.	.	.	.	.	.	.	.
75	.	.	.	.	.	.	.	.	.	.	.	.
76	.	14	.	.	.	.	.	.	.	.	.	.
77	.	.	.	.	.	.	.	.	.	.	.	.
78	.	11	.	.	.	.	.	.	.	.	.	.
79	.	11	.	.	19	.	.	.	.	.	.	.
80	.	.	.	.	.	.	.	.	.	.	.	.
81	.	.	.	.	.	.	.	.	.	.	.	.
82	.	.	.	.	.	.	.	.	.	.	.	.
83	.	.	.	.	.	.	.	.	.	.	.	.
P04722 77-93	.	.	.	.	.	.	.	.	.	.	.	.
P04722 77-93 E	.	.	.	.	.	.	.	.	.	.	.	.
P04722 77-93 E	.	.	.	.	.	.	.	.	.	.	.	.
P02863 77-93	.	.	.	.	.	.	.	11	.	.	.	.
P02863 77-93 E	.	.	.	.	.	.	.	.	.	.	.	.
Gliadin+C	***171***	40	25	16	10		18	14	.	17		***90***
Chymotrypsin	29	26	18	.	.	.	.	.	22	.	.	
Gliadin+Pepsin	***241***	151	29	24	48	.	16	45	.	19		***35***
Pepsin												

Table 17: tTG-deamidated gliadin peptide pools showing significant increase in IFN gamma responses between Day 0 and Day 6 of gluten challenge in HLA-DQ2 coeliac subjects C01-6 (Day 6 –Day 0 response, and ratio of responses to tTG-deamidated pool and same pool without tTG treatment)

Pool	IFNg ELISpot (Median sfc/million)	tTG: no tTG (Median)	Pool	IFNg ELISpot (Median sfc/million)	tTG: no tTG (Median)
9	59***	1.0	**49**	46***	1.4
10	116**	1.7	**50**	50***	4.6
11	24***	2.5	**51**	40***	1.7
12	133***	1.1	**52**	30***	3.1
13	26**	2.1	**53**	27**	1.4
42	30**	1.2	**76**	17***	1.1
43	32***	1.3	**79**	20***	0.9
44	24***	1.5	**80**	83***	1
45	10***	1.1	**81**	141***	1.1
46	12***	2.1	**82**	22***	1.5
48	17***	1.4	**83**	16**	1.8

Day 6 vs. Day 0 **P<0.02, ***P<0.01 by one-tailed Wilcoxon Matched-Pairs Signed-Ranks test

Table 18. Coeliac subjects: IFNγ ELISpot Responses >10 sfc/million PBMC and >4 x buffer only to tTG-treated Pepset Pools on Day 6 of gluten challenge (sfc/million PBMC) (*italic*: response also present on Day 0):

Group 1 – HLA-DQ2 (DQA1*0501-5, DQB1*0201/2),

Group 2 – HLA-DQ2/8 (DQA1*0501-5, *0301, and DQB1*0201/2, *0302), and

Group 3 – HLA-DQ8 (DQA1*0301, DQB1*0302) and absent or "incomplete" DQ2 (only DQA1*0501-5 or DQB1*0201/2)

	Group 1:						Group 2:			Group 3			
Subject	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12	C13
HLA-DQ	2, 6	2, 2	2, 5	2,5	2, 2	2, 2	2, 8	2, 8	2, 8	2, 8	6,8	7, 8	8, 8
Pool 1	.						23					223	
2	.											155	
3	.											41	
4	11									22	.		
5	.									.	.		
6	18			21			20	17		.	.		
7	.							353		.	.		
8	11	64				14	20	480		.	.		13
9	93	127		92	25		32	460		.	.		18
10	175	491	58	200	48		84	***787***		.	.		
11	32	118		33	14		26	27		12	.		
12	204	379	54	225	61		129	587		12	.		
13	93	142		29	18			***60***		.	.		11
14	.	45		21			17			.	.		
15	18	30					38	43		.	.		
16	.							37		.	.		
17	.									.	.		
18	.									.	.		
19	11									.	.		
20	11	215					51	167		.	.		
21	.								11	.	.		
22	.	21								.	.		
23	.	18		21						12	.		
24	.	15						10		.	.		
25	.	***15***								12	.		
26	.	***18***						13		12	.		
27	.	***15***								.	.		
28	.									.	.		11
29	.						11			.	.		
30	11						11			.	.		
31	.	70								.	.		
32	.	18					20			.	.		
33	11			10			***14***		***11***	.	40		***11***
34	.								11	.	.		
35	.									.	.		
36	.									.	.		
37	.			23		14				.	.		

38	.	24		19			20			.	.			
39	.	49		15					***11***	.	.			
40	.						14			.	.			
41	.	21								.	.			
42	39	42		44	21		11	63		12	.			
43	50	91	13	75	14		190	113		.	.	21		
44	32	97	17	96	13		87	107		.	.			
45	.	21	10	100	11		38	110		.	.			
46	14	55		102	18		63	***163***		.	.			
47	14	58		38			223	97		.	.	31		
48	21	106		60	14		144	353		.	.	57		
49	75	170	17	142	30		202	293		.	.	39		
50	57	***245***	23	140	61	***27***	248	***143***		.	.			11
51	68	106	10	127			220	***267***		.	.	29		
52	43	121		79	13	16	175	180		.	.			
53	36	94		92	29		69	53		.	.			
54	36			35	11		166	27		.	.	19	13	
55	.									.	.			
56	29								***11***	.	.			
57	.	36					20	13		.	.			
58	.									.	.			
59	.		10					53		.	.			
60	.	18		15			11	53		.	.			
61	.						20			.	.			
62	14	18		13			60			.	.			
63	.		10			14				28	.			
64	.	15								18	.			
65		36		25	***23***		35	27		.	***11***			
66				31	11	10	17			.	.			
67	.			17			17			.	.			
68	.		19	***127***		14				.	.			
69	.	15		10			***20***	20			.			
70	.	12	31		13	10				.				
71	11	21	13				14			.	18			
72	.					16				.	.			
73	.			13		***14***	11			.	.			
74	.	239					254	447		.	.			
75	.									.	.			
76	18	21	19	15						.	.	12		
77	.	88				10		13		.	.			
78	.	18	17	69						.	.			
79	11	85		44	29	12	44	43		.	.			
80	132	133	33	240	39	12	208	467		12	.	70		
81	171	318	113	367	104	12	211	530		.	.	74		
82	18	300	17	125	32	***16***	241	723		.	.			
83	14	164		31	21		163	277		15	.			
P04722 77-93	211	291	75	281	66		78	***740***		.	.			
P04722 77-93 E	164	297	108	221	64	10	84	653		.	.			
P04722 77-93 E	161	182	98	256	73	***16***	63	500		.	.			
P02863 77-93	139	164	35	94	36		29	603		.	.			
P02863 77-93 E	46	176	19	88	41		23	520		.	.			
Gliadin+C	***214***	***273***	***265***	***360***	***384***	***206***	***278***	***543***	17		***25***	***527***	71	
Chymotrypsin						***18***				.	.			
Gliadin+Pepsin	***239***	***315***	269	***406***	***207***	292	***357***	***557***		***42***	***89***	***335***	87	
Pepsin	.					14								

Table 19. Deamidated peptides with mean bioactivity > 10% of P04722 E85 (20 µg/ml) in HLA-DQ2 coeliac subjects C01-5

(Peptide Numbers in Table 19 correspond to Peptide Numbers and accompanying SEQ ID NOs. identified in Table 24)

Rank	No.	Sequence	Mean (SEM)	Rank	No.	Sequence	Mean (SEM)
	89	PQLPYPQPQLPYPQPQLPYP	94 (18)	**37**	413	SKQPQQPFPQPQQPQSTPQ	18 (4)
***2**	91	PQPFPQPQLPYPQPQLPYPQP	89 (12)	**38**	380	QPQQPQQPFPQPQQPQLPFP	18 (6)
***3**	74	MQLQPFPQPQLPYPQPQLPY	88 (14)	**39**	618	PQQSFSYQQQPFPQQPYPQQ	18 (7)
***4**	90	PQLPYPQPQLPYPQPQPFRP	87 (16)	***40**	78	LQLQPFPRPQLPYPQPQPFR	17 (8)
***5**	76	LQLQPFPQPQLPYPQPQPFR	85 (15)	**41**	390	QQTYPQRPQQPFPQTQQPQQ	17 (9)
6	626	PQQPQQPQQPFPQPQQPFPW	72 (23)	**42**	348	QQTFPQFQQTFPHQPQQQFP	16 (10)
7	627	QPFPQPQQPFPWQPQQPFPQ	66 (30)	**43**	409	QPQQPFPQLQQPQQPLPQPQ	16 (2)
***8**	631	FPQQPQQPFPQPQLPFPQQS	61 (12)	**44**	382	QQPFPQQPQQPFPQTQQPQQ	16 (6)
9	636	PQQPQQPFPQPQQPIPVQPQ	51 (10)	**45**	629	PFPQTQQSFPLQPQQPFPQQ	16 (5)
*10	73	LQLQPFPQPLQLPYPQPQLPY	49 (11)	**46**	643	PLQPQQPFPQQPQQPFPQQP	16 (6)
11	412	SQQPQQPFPQPQQQPFQPQQ	34 (19)	**47**	389	QQPFPQTQQPQQPFPQQPQQ	16 (6)
12	343	QQPQQPFPQPQQPQLPFPQQ	34 (11)	**48**	350	QQIFPQFQQTFPHQPQQAFP	15 (8)
***13**	68	LQLQPFPQPQLPYLQPQPFR	33 (10)	**49**	65	PFPSQQPYPQPQPFPQPQPF	15 (5)
***14**	66	LQLQPFPQPQLPYSQPQPFR	32 (7)	**50**	349	QQIFPQFPQQTFPHQPQQQFP	15 (9)
***15**	96	PQPFPPQLPYPQPQSFPPQQ	28 (6)	**51**	610	PWQQQPLPPQQSFSQQPFFS	15 (11)
16	393	QLPFPQPQQPFPQPQQPQQ	27 (8)	***52**	81	PQPQPFPPQLPYPQTQPFPP	15 (5)
17	355	QAFPQPQQTFPHQPQQQFPQ	27(15)	***53**	75	MQLQPFPQPQPFPPQLPYPQ	14 (5)
***18**	67	LQLQPFPQPLPYSQPQQFR	26 (6)	**54**	368	QQFPQPQQPQQPFPQQPQQQ	14 (7)
19	335	QQQQPFPQPQQPQQPFPQPQ	25 (11)	***55**	82	PQPQPFPQPQPFPPQLPYPQ	14 (3)
***20**	95	PQPFLPQLPYPQPQSFPPQQ	24 (6)	***56**	80	LQLQPFPQPQPFPPQLPYPQ	14 (4)
21	396	TQQPQQPFPQPQQPFPQTQ	23 (9)	**57**	624	FTQPQQPTPIQPQQPFPQQP	14 (6)
22	609	SCISGLERPWQQQFLPFQQS	23 (18)	**58**	407	QPQQPFPQSQQPQQPFPQPQ	14 (5)
23	385	QQPFPQPQQPQLPFPQQPQQ	23 (7)	**59**	337	QQQPFPQPQQPFCQQPQRTI	13 (4)
24	375	PQQPFPQPQQPQQPFPQPQQ	23 (10)	**60**	634	PQQLQQPFPLQPQQPFPQQP	13 (3)
25	406	QPQQPFPQLQQPQQPFPQPQ	22 (8)	**61**	388	QQPYPQQPQQPFPQTQQPQQ	13 (3)
26	625	PIQPQQPFPQQPQQPQQPFP	22 (9)	**62**	641	FPELQQPIPQQPQQPFPLQP	13 (7)
27	378	QQPQQPFPQQPQQQPFQPQQ	22 (10)	**63**	399	QQPFPQTQQPQQPFPQLQQP	13 (5)
28	371	PQQQFIQPQQPFPQQPQQTY	22 (10)	**64**	387	QQTFPQQPQLPFPQQPQQPF	13 (4)
29	642	PQQPQQPFPLQPQQPFPQQP	20 (8)	**65**	628	PFPWQPQQPFPQTQQSFPLQ	12 (4)
30	635	PLQPQQPFPQQPQQPFPQPQ	19 (5)	***66**	88	PQPFPPQLPYSQPQPFRPQQ	12 (3)
***31**	93	PQPFPPQLPYPQPQPFRPQQ	19 (5)	**67**	408	QPQQPFPQSKQPQQPFPQPQ	12 (5)
32	377	PQQQFPQPQQPQQPFPQPQ	19 (9)	***68**	77	LQLQPFPQPQPFPPQLPYPQ	11 (4)
33	411	LQQPQQPFPQPQQQLPQPQQ	19 (4)	**69**	370	PQQQFLQPQQPFPQQPQQPY	11 (5)
34	415	SQQPQQPFPQPQQPQQSFPQ	18 (5)	***70**	79	LQLQPFPQPQPFLPQLPYPQ	11 (5)
***35**	94	PQPFPPQLPYPQPPPFSPQQ	18 (3)	**71**	379	QQPQQQPFQPQQPQQPFPQP	11 (5)
36	329	PSGQVQWPQQQPFPQPQQPF	18 (4)	**72**	397	PQQPQQPFPQTQQPQQPFPQ	11 (3)

* Indicates homologue of A-gliadin 57-73 with the core sequence PQLP(Y/F)

Table 20. Peptides >10% as bioactive as P04722 QE65 grouped by structure. (Peptide Numbers in Table 20 correspond to Peptide Numbers and their accompanying SEQ ID NOs. identified in Table 24)

Rank	Peptide no. (Pool) Gliadin-subtype	Sequence	IFNg ELISpot response compared to P04722 77-93 QE85: mean (SEM)
Group 1: Homologues of A-gliadin 57-73			
	P04722 77-93	**QLQPFPQPQLPYPQPQP**	
1	89 (12) α	PQL...Y.....LPYP	94 (18)
2	91 (12) α	PQPFPPQL...Y.....	89 (12)
3	74 (10) α	M.....LPY	88 (14)
4	90 (12) α	PQL...Y.....PFRP	87 (16)
5	76 (10) α	L.....PFR	85 (15)
8	631 (81) ω	FPQQPQ.....F.....QS	61 (12)
10	73 (10) α	L.....LPY	49 (11)
13	68 (9) α	L.....L.....PFR	33 (10)
14	66 (9) α	L.....S.....PFR	32 (7)
18	67 (9) α	L.....S.....QFR	26 (6)
20	95 (13) α	PQPFL.....FPPQQ	24 (6)
31	93 (12) α	PQPFP.....PFRPQQ	19 (5)
35	94 (12) α	PQPFP.....PPFSPQQ	18 (3)
40	78 (10) α	L.....R.....PFR	17 (8)
52	81 (11) α	PQPQPFP.....T...PFPP	15 (5)
53	75 (10) α	MQLQPFPQPQPF.....	14 (5)
55	82 (11) α	PQPQPFPQPQPF.....	14 (3)
56	80 (10) α	LQLQPFPQPQPF.....	14 (4)
66	88 (11) α	PQPFP.....S.....PFRPQQ	12 (3)
68	77 (10) α	LQLQPFPQPQPFP.....	11 (4)
70	79 (10) α	LQLQPFPQPQPFL.....	11 (5)
Group 2: Homologues of peptide 626			
		QQPFPQPQQPFP	
6	626(80) ω	PQQPQQP.....W	72 (23)
7	627(80) ω	.....WQPQQPFPQ	66 (30)
9	636(81) ω	PQQP.....L...VQPQ	51 (10)
11	412(53) γ	SQQP.....Q.....PQQ	34 (19)
33	411(53) γ	LQQP.....Q.....PQQ	19 (4)
36	329(42) γ	PSGQVQWPQ.....	18 (4)
41	390(50) γ	QQTYPQRP.....T.....QQ	17 (9)
59	337(43) γ	Q.....CQQPQRTI	13 (4)
61	388(50) γ	QQPYPQQP.....T.....QQ	13 (3)
Group 3: Homologues of peptide 355			
		FPQPQQTFPHQPQQQFP	
17	355(46) γ	QA.....Q	27 (15)
42	348(45) γ	QQT.....	16 (10)
48	350(45) γ	QQI.....A.....	15 (8)
50	349(45) γ	QQI.....	15 (9)
Group 4: Homologues of Peptide 396			
		QQPFPQQPQQPFP	
21	396(51) γ	TQQP.....QTQ	23 (9)
27	378(49) γ	QQP.....QPQQ	22 (10)

28	371(48) γ	PQQQFIQP……TY	22 (10)
29	642(82) ω	PQQP……L……QQP	20 (8)
30	635(81) ω	PLQP……QPQ	19 (5)
44	382(49) γ	……QTQQPQQ	16 (6)
45	629(81) ω	PFPQT……S……L……QQ	16 (5)
46	643(82) ω	PLQP……QQP	16 (6)
60	634(81) ω	PQQL……L……QQP	13 (3)
64	387(50) γ	……T……L……QQPQQPF	13 (4)
62	641(82) ω	FPEL……I……LQP	13 (7)
		Group 5: Homologues of Peptide 343 (overlap Groups 2 and 4)	
		QQPFPQPQQPQLPFPQ	
12	343(44) γ	QQP……Q	34 (11)
16	393(51) γ	QLPFPQQP……	27 (8)
19	335(43) γ	QQ……Q……PQ	25 (11)
23	385(50) γ	……QPQQ	23 (7)
24	375(48) γ	P……Q……PQQ	23 (10)
25	406(52) γ	QP……L……Q……PQ	22 (8)
32	377(49) γ	P……Q……Q……QPQ	19 (9)
34	415(53) γ	SQQP……QS……	18 (5)
37	413(53) γ	SKQP……QS……	18 (4)
38	380(49) γ	QPQQP……	18 (6)
43	409(53) γ	QP……L……Q…L……PQ	16 (2)
47	389(50) γ	……T……Q……QPQQ	16 (6)
58	407(52) γ	QP……S……Q……PQ	14 (5)
63	399(51) γ	……T……Q……LQQP	13 (5)
67	408(52) γ	QP……SK……Q……PQ	12 (5)
71	379(49) γ	QQP……Q……Q……P	11 (5)
72	397(51) γ	PQQP……T……Q……	11 (3)
		Group 6: Peptide 625	
		PIQPQQPFPQQP	
26	625(80) ω	**……QQPQQPFP**	22 (9)
57	624(80) ω	FTQPQQPT……	14 (6)
65	628(80) ω	PF…W……TQQSFPLQ	12 (4)
		Group 7: Peptide 618	
39	618(79) ω	**PQQSFSYQQQPFPQQPYPQQ**	18 (7)

Table 21. Bioactivity of individual tTG-deamidated Pools 1-3 peptides in Subject C12: (Peptide Numbers in Table 21 correspond to Peptide Numbers and their accompanying SEQ ID NOs. identified in Table 24)

No.	Sequence	%	No.	Sequence	%
8	AVRWPVPQLQPQNPSQQQPQ	100	23	LQPQNPSQQQPQEQVPLMQQ	26
		85			18
5	MVRVTVPQ.....		14	.....EQVPLVQQ	
		82			18
6	AVRVSVPQ.....		15	.....H.....EQVPLVQQ	
		77			18
3	MVRVPVPQ.....H.....		17	.....KQVPLVQQ	
		67			13
1	AVRFPVPQ.....L.....		16	.....D.....EQVPLVQQ	
		59			8
2	MVRVPVPQ.....		13	.....EQVPLVQQ	
		49			5
9	AVRVPVPQ.....L.....		22	.....K.....EQVPLVQQ	
		49			3
7	AVRVPVPQ.....		18	.....L.....EQVPLVQE	
		33			3
10	MVRVPVPQ.....L.....		19	.....L.....EQVPLVQE	
4	MVRVPMPQ.....D.....	15	20	P.....P.....GQVPLVQQ	0
12	AVRVPVPQ.....K.....	8	21	P.....P.....RQVPLVQQ	0
11	AVRVPVPQP.....P.....	0			

Core sequence of epitope is underlined. Predicted deamidated sequence is: LQPENPSQEQPE

Table 22: Phylogenetic groupings of wheat (Triticum aestivum) gliadins

	Alpha/beta-gliadins (n=61)		
A1a1	AAA96525, EEWTA, P02863	A1b13	B22364, P04271
A1a2	CAB76963	A2a1	AAB23109, CAA35238, P18573, S10015
A1a3	AAA96276	A2a2	CAB76964
A1a4	CAA26384, S07923	A2b1	P04724, T06500, AAA348282
A1a5	AAA34280	A2b2	D22364
A1a6	P04728	A2b3	P04722, T06498, AAA34276
A1b1	CAB76962	A2b4	C22364
A1b2	CAB76961	A2b5	CAB76956
A1b3	BAA12318	A3a1	AAA34277, CAA26383, P04726, S07361
A1b4	CAB76960	A3a2	1307187B, A27319, S13333
A1b5	CAB76958	A3b1	AAA96522
A1b6	CAB76959	A3b2i	AAA34279, P04727,
A1b7	CAB76955	A3b2ii	CAA26385, S07924
A1b8	AAA96524	A3b3	A22364, AAA34278, AAB23108, C61218, P04725
A1b9	CAA10257	A4a	P04723, AAA34283, T06504
A1b10	AAA96523, T06282	A4b	E22364
A1b11	AAA17741, S52124	A4c	CAB76957
A1b12	AAA34281	A4d	CAB76954
	Gamma-gliadins (n=47)		Gamma-gliadins
Gl1a	P08079, AAA34288, PS0094, CAC11079, AAD30556, CAC11057, CAC11065, CAC11056	Gl5a	AAK84774, AAK84772
Gl1b	CAC11089, CAC11064, CAC11080, CAC11078, AAD30440	Gl5b	AAK84773
Gl1c	CAC11087	Gl5c	AAK84776
Gl1d	CAC11088	Gl6a	JA0153, P21292, AAA34272, 1507333A
Gl1e	CAC11055	Gl6b	AAK84777
Gl2a	JS0402, P08453, AAA34289	Gl6c	1802407A, AAK84775, AAK84780
Gl2b	AAF42989, AAK84779, AAK84779	Gl7	AAB31090
Gl3a	AAK84778	GIIa	AAA34287, P04730, S07398
Gl3b	CAB75404	GIIb	1209306A
Gl3c	BAA11251	GIII1a	P04729
Gl4	EEWTG, P06659, AAA34274	GIII1b	AAA34286
	Omega-gliadins (n=3)		
O1a	AAG17702		
O1b	P02865		
O1c	A59156		

Table 23. Synthetic peptides spanning all known wheat gliadin 12mers (Peptide Numbers 1 through 652 correspond to SEQ ID NO:107 through SEQ ID NO:758, respectively)

Protein	Position*	Sequence	No.
POOL 1			
A1A1	20 AVRF	PVPQ LQPQ NPSQ QLPQ	1
A1A2	20 MVRV	PVPQ LQPQ NPSQ QQPQ	2
A1B1	20 MVRV	PVPQ LQPQ NPSQ QHPQ	3
A1B2	20 MVRV	PMPQ LQPQ DPSQ QQPQ	4
A1B7	20 MVRV	TVPQ LQPQ NPSQ QQPQ	5
A1B8	20 AVRV	SVPQ LQPQ NPSQ QQPQ	6
A1B8	20 AVRV	PVPQ LQPQ NPSQ QQPQ	7
A1B10	20 AVRW	PVPQ LQPQ NPSQ QQPQ	8
POOL 2			
A2B3	20 AVRV	PVPQ LQLQ NPSQ QQPQ	9
A2B5	20 MVRV	PVPQ LQLQ NPSQ QQPQ	10
A3A1	20 AVRV	PVPQ PQPQ NPSQ PQPQ	11
A3B1	20 AVRV	PVPQ LQPK NPSQ QQPQ	12
A1A1	28 LQPQ	NPSQ QLPQ EQVP LVQQ	13
A1A2	28 LQPQ	NPSQ QQPQ EQVP LVQQ	14
A1B1	28 LQPQ	NPSQ QHPQ EQVP LVQQ	15
A1B2	28 LQPQ	DPSQ QQPQ EQVP LVQQ	16
POOL 3			
A2B1	28 LQPQ	NPSQ QQPQ KQVP LVQQ	17
A2B3	28 LQLQ	NPSQ QQPQ EQVP LVQE	18
A2B5	28 LQLQ	NPSQ QQPQ EQVP LVQE	19
A3A1	28 PQPQ	NPSQ PQPQ EQVP LVQQ	20
A3A2	28 PQPQ	NPSQ PQPQ RQVP LVQQ	21
A3B1	28 LQPK	NPSQ QQPQ EQVP LVQQ	22
A4A	28 LQPQ	NPSQ QQPQ EQVP LMQQ	23
A1A1	36 QLPQ	EQVP LVQQ QQFL GQQQ	24
POOL 4			
A1B1	36 QHPQ	EQVP LVQQ QQFL GQQQ	25
A1B2	36 QQPQ	EQVP LVQQ QQFL GQQQ	26
A1B12	36 QQPQ	EQVP LVQQ QQFL GQQQ	27
A2A1	36 QQPQ	EQVP LVQQ QQEP GQQQ	28
A2B1	36 QQPQ	KQVP LVQQ QQFP GQQQ	29
A2B3	36 QQPQ	EQVP LVQE QQFQ GQQQ	30
A3A1	36 PQPQ	GQVP LVQQ QQFP GQQQ	31
A3A2	36 PQPQ	RQVP LVQQ QQFP GQQQ	32
POOL 5			
A4A	36 QQPQ	EQVP LMQQ QQFL PGQQ	33
A1A1	44 LVQQ	QQFL GQQQ PFPP QQPY	34
A1B1	44 LVQQ	QQFL GQQQ SFPP QQPY	35
A1B12	44 LVQQ	QQFL GQQQ PFPP QQPY	36
A2A1	44 LVQQ	QQFP GQQQ PFPP QQPY	37
A2B3	44 LVQE	QQFQ GQQQ PFPP QQPY	38
A3A1	44 LVQQ	QQFP GQQQ QFPP QQPY	39
A4A	44 LMQQ	QQQF PGQQ EQFP PQQP	40
POOL 6			
A4D	44 LMQQ	QQQF PGQQ ERFP PQQP	41
A1A1	53 GQQQ	PFPP QQPY PQPQ PFPS	42
A1A3	53 GQQQ	PFPP QQPY PQPQ FPSQ	43
A1B1	53 GQQQ	SFPP QQPY PQPQ PFPS	44
A2B1	53 GQQQ	PFPP QQPY PQQQ PFPS	45
A3A1	53 GQQQ	QFPP QQPY PQPQ PFPS	46
A4A	53 GQQE	QFPP QQPY PHQQ PFPS	47
A4D	53 GQQE	RFPP QQPY PHQQ PFPS	48
POOL 7			
A1A1	61 QQPY	PQPQ PFPS QLPY LQLQ	49
A1A3	61 QQPY	PQPQ FPSQ LPYL QLQP	50
A1B1	61 QQPY	PQPQ PFPS QQPY LQLQ	51
A2B1	61 QQPY	PQQQ PFPS QQPY MQLQ	52
A4A	61 QQPY	PHQQ PFPS QQPY PQPQ	53
A1A1	69 PFPS	QLPY LQLQ PFPQ PQLP	54
A1B1	69 PFPS	QQPY LQLQ PFPQ PQLP	55
POOL 43			
GI2A	33 QQQL	VPQL QQPL SQQP QQTF	331
GI3A	33 QQQP	FPQP HQPF SQQP QQTF	332
GI4	33 QQQP	FLQP HQPF SQQP QQIF	333
GI5A	33 QQQQ	PFPQ PQQP FSQQ PQQI	334
GI5B	33 QQQQ	PFPQ PQQP QQPF PQPQ	335
GI5C	33 QQQP	FRQP QQPF YQQP QHTF	336
GI6A	33 QQQP	FPQP QQPF CQQP QRTI	337
GI6C	42 QQQP	FPQP QQPF CEQP QRTI	338
POOL 44			
GI1A	42 HQPF	SQQP QQTF PQPQ QTFP	339
GI2A	42 QQPL	SQQP QQTF PQPQ QTFP	340
GI4	42 HQPF	SQQP QQIF PQPQ QTFP	341
GI5A	42 QQPF	SQQP QQIF PQPQ QTFP	342
GI5B	42 QQPQ	QFPP QPQQ PQLP FPQQ	343
GI5C	42 QQPF	YQQP QHTF PQPQ QTCP	344
GI6A	42 QQPF	CQQP QRTI PQPH QTFH	345
GI6B	42 QQPF	CQQP QQTI PQPH QTFH	346
POOL 45			
GI6C	42 QQPF	CEQP QRTI PQPH QTFH	347
GI1A	50 QQIF	PQPQ QTFP HQPQ QQFP	348
GI4	50 QQIF	PQPQ QTFP HQPQ QQFP	349
GI5A	50 QQIF	PQPQ QTFP HQPQ QAFP	350
GI6A	50 QRTI	PQPH QTFH HQPQ QTFP	351
GI5A	58 QTFP	HQPQ QAFP QPQQ TFPH	352
GI6A	58 QTFH	HQPQ QTFP QPQQ TYPH	353
GI6C	58 QTFH	HQPQ QTFP QPEQ TYPH	354
POOL 46			
GI5A	66 QAFP	QPQQ TFPH QPQQ QFPQ	355
GI5C	66 QHTF	PQPQ QTCP HQPQ QQFP	356
GI6A	66 QTFP	QPQQ TYPH QPQQ QFPQ	357
GI6C	66 QTFP	QPEQ TYPH QPQQ QFPQ	358
GI1A	73 QTFP	HQPQ QQFP QPQQ PQQQ	359
GI2A	73 QTFP	HQPQ QQVP QPQQ PQQP	360
GI3A	73 QTFP	HQPQ QQFS QPQQ PQQQ	361
GI5C	73 QTCP	HQPQ QQFP QPQQ PQQP	362
POOL 47			
GI6A	73 QTYP	HQPQ QQFP QTQQ PQQP	363
GI1A	81 QQFP	QPQQ PQQQ FLQP QQPF	364
GI2A	81 QQVP	QPQQ PQQP FLQP QQPF	365
GI3A	81 QQFS	QPQQ PQQQ FIQP QQPF	366
GI4	81 QQFP	QPQQ PQQQ FLQP RQPF	367
GI5A	81 QQFP	QPQQ PQQP FPQQ PQQQ	368
GI6A	81 QQFP	QTQQ PQQP FPQP QQTF	369
GI1A	89 PQQQ	FLQP QQPF PQQP QQPY	370
POOL 48			
GI3A	89 PQQQ	FIQP QQPF PQQP QQTY	371
GI3B	89 PQQQ	FIQP QQPQ QTYP QRPQ	372
GI4	89 PQQQ	FLQP RQPF PQQP QQPY	373
GI5A	89 PQQP	FPQQ PQQQ FPQP QQPQ	374
GI5C	89 PQQP	FPQP QQPQ QPFP QPQQ	375
GI6A	89 PQQP	FPQP QQTF PQQP QLPF	376
POOL 49			
GI5A	97 PQQQ	FPQP QQPQ QPFP QQPQ	377
GI5A	105 QQPQ	QPFP QQPQ QQFP QPQQ	378
GI5A	113 QQPQ	QQFP QPQQ PQQP FPQP	379
GI5A	121 QPQQ	PQQP FPQP QQPQ LPFP	380
GI1A	126 QQPF	PQQP QQPY PQQP QQPF	381
GI2A	126 QQPF	PQQP QQPF PQTQ QPQQ	382
GI3A	126 QQPF	PQQP QQTY PQRP QQPF	383
GI4	126 RQPF	PQQP QQPY PQQP QQPF	384
POOL 50			

A1B10 69 PFPS QQPY LQLQ PFSQ PQLP
POOL 8
A1B11 69 PFPS QQPY LQLQ PFLQ PQLP
A1B12 69 PFPS QQPY LQLQ PFLQ PQPF
A2A1 69 PFPS QQPY LQLQ PFPQ PQLP
A2B1 69 PFPS QQPY MQLQ PFPQ PQLP
A2B2 69 PFPS QQPY MQLQ PFPQ PQPF
A2B4 69 PFPS QQPY LQLQ PFPQ PQPF
A2B5 69 PFPS QQPY LQLQ PFPR PQLP
A4A 69 PFPS QQPY PQPQ PFPP QLPY
POOL 9
A4B 69 PFPS QQPY PQPQ PFPQ PQPF
A1A1 77 LQLQ PFPQ PQLP YSQP QPFR
A1A4 77 LQLQ PFPQ PQLP YSQP QQFR
A1B1 77 LQLQ PFPQ PQLP YLQP QPFR
A1B4 77 LQLQ PFPQ PQLS YSQP QPFR
A1B10 77 LQLQ PFSQ PQLP YSQP QPFR
A1B11 77 LQLQ PFLQ PQLP YSQP QPFR
A1B12 77 LQLQ PFLQ PQPF PPQL PYSQ
POOL 10
A2A1 77 LQLQ PFPQ PQLP YPQP QLPY
A2B1 77 MQLQ PFPQ PQLP YPQP QLPY
A2B2 77 MQLQ PFPQ PQPF PPQL PYPQ
A2B3 77 LQLQ PFPQ PQLP YPQP QPFR
A2B4 77 LQLQ PFPQ PQPF PPQL PYPQ
A2B5 77 LQLQ PFPR PQLP YPQP QPFR
A3B1 77 LQLQ PFPQ PQPF LPQL PYPQ
A3B3 77 LQLQ PFPQ PQPF PPQL PYPQ
POOL 11
A4A 77 PQPQ PFPP QLPY PQTQ PFPP
A4B 77 PQPQ PFPQ PQPF PPQL PYPQ
A1A1 85 PQLP YSQP QPFR PQQP YPQP
A1A6 85 PQLP YSQP QQFR PQQP YPQP
A1B1 85 PQLP YLQP QPFR PQQP YPQP
A1B4 85 PQLS YSQP QPFR PQQP YPQP
A1B6 85 PQLS YSQP QPFR PQQL YPQP
A1B12 85 PQPF PPQL PYSQ PQPF RPQQ
POOL 12
A2A1 85 PQLP YPQP QLPY PQPQ LPYP
A2B1 85 PQLP YPQP QLPY PQPQ PFRP
A2B2 85 PQPF PPQL PYPQ PQLP YPQP
A2B3 85 PQLP YPQP QPFR PQQP YPQP
A2B4 85 PQPF PPQL PYPQ PQPF RPQQ
A3A1 85 PQPF PPQL PYPQ PPPF SPQQ
POOL 13
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A3B3 85 PQPF PPQL PYPQ PQSF PPQQ
A4A 85 QLPY PQTQ PFPP QQPY PQPQ
A4B 85 PQPF PPQL PYPQ TQPF PPQQ
A2A1 106 LPYP QPQP FRPQ QPYP QSQP
A2B1 106 LPYP QPQP FRPQ QSYP QPQP
A3A1 106 LPYP QPPP FSPQ QPYP QPQP
A3B1 106 LPQL PYPQ PQSF PPQQ PYPQ
POOL 14
A4A 106 PPQL PYPQ TQPF PPQQ PYPQ
A1A1 112 QPFR PQQP YPQP QPQY SQPQ
A1B6 112 QPFR PQQL YPQP QPQY SQPQ
A2A1 112 QPFR PQQP YPQP QPQY SQPQ
A2B1 112 QPFR PQQS YPQP QPQY SQPQ
A3A1 112 PPFS PQQP YPQP QPQY PQPQ
A3B1 112 QSFP PQQP YPQQ RPKY LQPQ
A3B2 112 QSFP PQQP YPQQ RPMY LQPQ
POOL 15
A3B3 112 QSFP PQQP YPQQ QPQY LQPQ
A4A 112 QPFP PQQP YPQP QPQY PQPQ
A1A1 120 YPQP QPQY SQPQ QPIS QQQQ
A1B3 120 YPQP QPQY SQPQ EPIS QQQQ
A2A1 120 YPQS QPQY SQPQ QPIS QQQQ
A3A1 120 YPQP QPQY PQPQ QPIS QQQA
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GI5C 126 QQPF PQPQ QAQL PFPQ QPQQ 386
GI6A 126 QQTF PQQP QLPF PQQP QQPF 387
GI1A 134 QQPY PQQP QQPF PQTQ QPQQ 388
GI2A 134 QQPF PQTQ QPQQ PFPQ QPQQ 389
GI3A 134 QQTY PQRP QQPF PQTQ QPQQ 390
GI5A 134 QPQL PFPQ QPQQ QPQQ PFPQ 391
GI5C 134 QAQL PFPQ QPQQ PLPQ PQQP 392
POOL 51
GI6A 134 QLPF PQQP QQPF PQPQ QPQQ 393
GI2A 142 QPQQ PFPQ QPQQ PFPQ TQQP 394
GI2A 150 QPQQ PFPQ TQQP QQPF PQQP 395
GI2A 158 TQQP QQPF PQQP QQPF PQTQ 396
GI2A 166 PQQP QQPF PQTQ QPQQ PFPQ 397
GI8A 170 QQPF PQTQ QPQQ LFPQ SQQP 398
GI2A 170 QQPF PQTQ QPQQ PFPQ LQQP 399
GI3A 170 QQPF PQTQ QPQQ PFPQ SQQP 400
POOL 52
GI4 170 QQPF PQTQ QPQQ PFPQ SKQP 401
GI5A 170 QQPF PQPQ QPQQ PFPQ LQQP 402
GI5C 170 QQPL PQPQ QPQQ PFPQ SQQP 403
GI6A 170 QQPF PQTQ QPQQ PFPQ SQQP 404
GI1A 178 QPQQ LFPQ SQQP QQQF SQPQ 405
GI2A 178 QPQQ PFPQ LQQP QQPF PQPQ 406
GI3A 178 QPQQ PFPQ SQQP QQPF PQPQ 407
GI4 178 QPQQ PFPQ SKQP QQPF PQPQ 408
POOL 53
GI5A 178 QPQQ PFPQ LQQP QQPL PQPQ 409
GI1A 186 SQQP QQQF SQPQ QQFP QPQQ 410
GI2A 186 LQQP QQPF PQPQ QQLP QPQQ 411
GI2A 186 SQQP QQPF PQPQ QQFP QPQQ 412
GI4 186 SKQP QQPF PQPQ QPQQ SFPQ 413
GI5A 186 LQQP QQPL PQPQ QPQQ PFPQ 414
GI5C 186 SQQP QQPF PQPQ QPQQ SFPQ 415
GI1A 194 SQPQ QQFP QPQQ PQQS FPQQ 416
POOL 54
GI2A 194 PQPQ QQLP QPQQ PQQS FPQQ 417
GI3A 194 PQPQ QQFP QPQQ PQQS FPQQ 418
GI4 194 PQPQ QPQQ SFPQ QQPS LIQQ 419
GI5A 194 PQPQ QPQQ PFPQ QQQP LIQP 420
GI5C 194 PQPQ QPQQ SFPQ QQQP LIQP 421
GI1A 202 QPQQ PQQS FPQQ QPPF IQPS 422
GI2A 202 QPQQ PQQS FPQQ QRPF IQPS 423
GI3A 202 QPQQ PQQS FPQQ QPSL IQQS 424
POOL 55
GI1A 210 FPQQ QPPF IQPS LQQQ VNPC 425
GI2A 210 FPQQ QRPF IQPS LQQQ LNPC 426
GI3A 210 FPQQ QPSL IQQS LQQQ LNPC 427
GI5A 210 FPQQ QQPL IQPY LQQQ MNPC 428
GI6A 210 FPQQ QQPA IQSF LQQQ MNPC 429
GI1A 218 IQPS LQQQ VNPC KNFL LQQC 430
GI2A 218 IQPS LQQQ LNPC KNFL LQQS 431
GI3A 218 IQQS LQQQ LNPC KNFL LQQC 432
POOL 56
GI5A 218 IQPY LQQQ MNPC KNYL LQQC 433
GI6A 218 IQSF LQQQ MNPC KNFL LQQC 434
GI1A 226 VNPC KNFL LQQC KPVS LVSS 435
GI2A 226 LNPC KNIL LQQS KPAS LVSS 436
GI3A 226 LNPC KNFL LQQC KPVS LVSS 437
GI5A 226 MNPC KNYL LQQC NPVS LVSS 438
GI6A 226 MNPC KNFL LQQC NHVS LVSS 439
GI1A 234 LQQC KPVS LVSS LWSM IWPQ 440
POOL 57
GI2A 234 LQQS KPAS LVSS LWSI IWPQ 441
GI3A 234 LQQC KPVS LVSS LWSM ILPR 442
GI5A 234 LQQC NPVS LVSS LVSM ILPR 443
GI6A 234 LQQC NHVS LVSS LVSI ILPR 444
GI1A 242 LVSS LWSM IWPQ SDCQ VMRQ 445
GI2A 242 LVSS LWSI IWPQ SDCQ VMRQ 446

A3B1 120 YPQQ RPKV LQPQ QPIS QQQA
A3B2 120 YPQQ RPMY LQPQ QPIS QQQA
POOL 16
A3B3 120 YPQQ QPQY LQPQ QPIS QQQA
A1A1 128 SQPQ QPIS QQQQ QQQQ QQQQ
A1B3 128 SQPQ EPIS QQQQ QQQQ QQQI
A3A1 128 PQPQ QPIS QQQA QQQQ QQQQ
A1A1 138 QQQQ QQQQ QQQQ QQQQ ILQQ
A1A6 138 QQQQ QQQQ QQQQ QEQQ ILQQ
A1B11 138 QQQQ QQQQ QQQQ QQQQ IIQQ
A2A1 138 QQQQ QQQQ QQKQ QQQQ QQQI
POOL 17
A4B 139 AQQQ QQQQ QQQQ QQQQ TLQQ
A1A1 146 QQQQ QQQQ ILQQ ILQQ QLIP
A1A6 146 QQQQ QEQQ ILQQ ILQQ QLIP
A1B6 146 QQQQ QEQQ ILQQ MLQQ QLIP
A1B10 146 QQQQ QEQQ ILQQ ILQQ QLTP
A1B11 146 QQQQ QQQQ IIQQ ILQQ QLIP
A2A1 146 QQKQ QQQQ QQQI LQQI LQQQ
A3A2 146 QQQQ QQQQ ILPQ ILQQ QLIP
POOL 18
A4A 146 QQQQ QQQQ TLQQ ILQQ QLIP
A1A1 163 ILQQ ILQQ QLIP CMDV VLQQ
A1B6 163 ILQQ MLQQ QLIP CMDV VLQQ
A1B10 163 ILQQ ILQQ QLTP CMDV VLQQ
A2B1 163 ILQQ ILQQ QLIP CRDV VLQQ
A3A2 163 ILPQ ILQQ QLIP CRDV VLQQ
A4A 163 TLQQ ILQQ QLIP CRDV VLQQ
A1A1 171 QLIP CMDV VLQQ HNIA HGRS
POOL 19
A1A3 171 QLIP CMDV VLQQ HNKA HGRS
A1B2 171 QLIP CMDV VLQQ HNLA HGRS
A1B7 171 QLIP CMDV VLQQ HNIV HGRS
A1B10 171 QLTP CMDV VLQQ HNIA RGRS
A1B11 171 QLIP CMDV VLQQ HNIV HGKS
A2A1 171 QLIP CRDV VLQQ HSIA YGSS
A2B1 171 QLIP CRDV VLQQ HSIA HGSS
A2B3 171 QLIP CRDV VLQQ HNIA HGSS
POOL 20
A3A1 171 QLIP CRDV VLQQ HNIA HARS
A3B1 171 QLIP CRDV VLQQ HNIA HASS
A1A1 179 VLQQ HNIA HGRS QVLQ QSTY
A1A3 179 VLQQ HNKA HGRS QVLQ QSTY
A1B2 179 VLQQ HNLA HGRS QVLQ QSTY
A1B7 179 VLQQ HNIV HGRS QVLQ QSTY
A1B10 179 VLQQ HNIA RGRS QVLQ QSTY
A1B11 179 VLQQ HNIV HGKS QVLQ QSTY
POOL 21
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A2B1 179 VLQQ HSIA HGSS QVLQ QSTY
A2B3 179 VLQQ HNIA HGSS QVLQ ESTY
A3A1 179 VLQQ HNIA HARS QVLQ QSTY
A3B1 179 VLQQ HNIA HASS QVLQ QSTY
A4A 179 VLQQ HNIA HASS QVLQ QSSY
A1A1 187 HGRS QVLQ QSTY QLLQ ELCC
A1A3 187 HGRS QVLQ QSTY QLLR ELCC
POOL 22
A1B8 187 HGRS QVLQ QSTY QLLR ELCC
A1B11 187 HGKS QVLQ QSTY QLLQ ELCC
A2A1 187 YGSS QVLQ QSTY QLVQ QLCC
A2B1 187 HGSS QVLQ QSTY QLVQ QFCC
A2B3 187 HGSS QVLQ ESTY QLVQ QLCC
A3A1 187 HARS QVLQ QSTY QPLQ QLCC
A3B1 187 HASS QVLQ QSTY QLLQ QLCC
A4A 187 HASS QVLQ QSSY QQLQ QLCC
POOL 23
A1A1 195 QSTY QLLQ ELCC QHLW QIPE
A1A3 195 QSTY QLLR ELCC QHLW QIPE
A1B8 195 QSTY QLLR ELCC QHLW QIPE

GI3A 242 LVSS LWSM ILPR SDCQ VMRQ 117 447
GI4 242 LVSS LWSI ILPP SDCQ VMRQ 118 448
POOL 58 .
GI5A 242 LVSS LVSM ILPR SDCK VMRQ 119 449
GI5C 242 LVSS LVSM ILPR SDCQ VMQQ 120 450
GI6A 242 LVSS LVSI ILPR SDCQ VMQQ 121 451
GI1A 250 IWPQ SDCQ VMRQ QCCQ QLAQ 122 452
GI3A 250 ILPR SDCQ VMRQ QCCQ QLAQ 123 453
GI4 250 ILPP SDCQ VMRQ QCCQ QLAQ 124 454
GI5A 250 ILPR SDCK VMRQ QCCQ QLAR 125 455
GI5C 250 ILPR SDCQ VMQQ QCCQ QLAQ 126 456
POOL 59 .
GI1A 258 VMRQ QCCQ QLAQ IPQQ LQCA 127 457
GI5A 258 VMRQ QCCQ QLAR IPQQ LQCA 128 458
GI5C 258 VMQQ QCCQ QLAQ IPRQ LQCA 129 459
GI6A 258 VMQQ QCCQ QLAQ IPQQ LQCA 130 460
GI1A 266 QLAQ IPQQ LQCA AIHT IIHS 131 461
GI1B 266 QLAQ IPQQ LQCA AIHT VIHS 132 462
GI2A 266 QLAQ IPQQ LQCA AIHS VVHS 133 463
GI3A 266 QLAQ IPQQ LQCA AIHS IVHS 134 464
POOL 60 .
GI5A 266 QLAR IPQQ LQCA AIHG IVHS 135 465
GI5C 266 QLAQ IPRQ LQCA AIHS VVHS 136 466
GI6A 266 QLAQ IPQQ LQCA AIHS VAHS 137 467
GI1A 274 LQCA AIHT IIHS IIMQ QEQQ 138 468
GI1B 274 LQCA AIHT VIHS IIMQ QEQQ 139 469
GI2A 274 LQCA AIHS VVHS IIMQ QQQQ 140 470
POOL 61 141 .
GI3A 274 LQCA AIHS IVHS IIMQ QEQQ 142 471
GI4 274 LQCA AIHS VVHS IIMQ QEQQ 472 .
GI5A 274 LQCA AIHG IVHS IIMQ QEQQ 143 473
GI6A 274 LQCA AIHS VAHS IIMQ QEQQ 144 474
GI1A 282 IIHS IIMQ QEQQ EQQQ GMHI 145 475
GI1B 282 VIHS IIMQ QEQQ QGMH ILLP 146 476
GI2A 282 VVHS IIMQ QQQQ QQQQ QGID 147 477
GI3A 282 IVHS IIMQ QEQQ EQRQ GVQI 148 478
POOL 62 149 .
GI4 282 VVHS IIMQ QEQQ EQLQ GVQI 150 479
GI5A 282 IVHS IIMQ QEQQ QQQQ QQQQ 480 .
GI5C 282 VVHS IVMQ QEQQ QGIQ ILRP 151 481
GI6A 282 VAHS IIMQ QEQQ QGVP ILRP 152 482
GI1A 290 QEQQ EQQQ GMHI LLPL YQQQ 153 483
GI2A 290 QQQQ QQQQ QGID IFLP LSQH 154 484
GI2B 290 QQQQ QQQQ QGMH IFLP LSQQ 155 485
GI3A 290 QEQQ EQRQ GVQI LVPL SQQQ 156 486
POOL 63 157 .
GI4 290 QEQQ EQLQ GVQI LVPL SQQQ 158 487
GI5A 290 QEQQ QQQQ QQQQ QQQG IQIM 488 .
GI5C 290 QEQQ QGIQ ILRP LFQL VQGQ 159 489
GI6A 290 QEQQ QGVP ILRP LFQL AQGL 160 490
GI5A 298 QQQQ QQQG IQIM RPLF QLVQ 161 491
GI1A 305 GMHI LLPL YQQQ QVGQ GTLV 162 492
GI2A 305 GIDI FLPL SQHE QVGQ GSLV 163 493
GI2B 305 GMHI FLPL SQQQ QVGQ GSLV 164 494
POOL 64 165 .
GI3A 305 GVQI LVPL SQQQ QVGQ GTLV 166 495
GI4 305 GVQI LVPL SQQQ QVGQ GILV 496 .
GI5A 305 GIQI MRPL FQLV QGQG IIQP 167 497
GI5C 305 GIQI LRPL FQLV QGQG IIQP 168 498
GI6A 305 GVPI LRPL FQLA QGLG IIQP 169 499
GI1A 313 YQQQ QVGQ GTLV QGQG IIQP 170 500
GI2A 313 SQHE QVGQ GSLV QGQG IIQP 171 501
GI2B 313 SQQQ QVGQ GSLV QGQG IIQP 172 502
POOL 65 173 .
GI3A 313 SQQQ QVGQ GTLV QGQG IIQP 174 503
GI4 313 SQQQ QVGQ GILV QGQG IIQP 504 .
GI1A 321 GTLV QGQG IIQP QQPA QLEA 175 505
GI2A 321 GSLV QGQG IIQP QQPA QLEA 176 506
GI5A 321 FQLV QGQG IIQP QQPA QLEV 177 507

A2A1 195 QSTY QLVQ QLCC QQLW QIPE
A2B1 195 QSTY QLVQ QFCC QQLW QIPE
A3A1 195 QSTY QPLQ QLCC QQLW QIPE
A3B1 195 QSTY QLLQ QLCC QQLL QIPE
A4A 195 QSSY QQLQ QLCC QQLF QIPE
POOL 24
A1A1 203 ELCC QHLW QIPE QSQC QAIH
A1B6 203 ELCC QHLW QILE QSQC QAIH
A1B10 203 ELCC QHLW QIPE KLQC QAIH
A2A1 203 QLCC QQLW QIPE QSRC QAIH
A2B1 203 QFCC QQLW QIPE QSRC QAIH
A3B1 203 QLCC QQLL QIPE QSRC QAIH
POOL 25
A3B3 203 GLCC QQLL QIPE QSQC QAIH
A4A 203 QLCC QQLF QIPE QSRC QAIH
A1A1 211 QIPE QSQC QAIH NVVH AIIL
A1B3 211 QIPE QSQC QAIQ NVVH AIIL
A1B6 211 QILE QSQC QAIH NVVH AIIL
A1B9 211 QIPE QSQC QAIH KVVH AIIL
A1B10 211 QIPE KLQC QAIH NVVH AIIL
A2A1 211 QIPE QSRC QAIH NVVH AIIL
POOL 26
A3B3 211 QIPE QSQC QAIH NVAH AIIM
A4A 211 QIPE QSRC QAIH NVVH AIIL
A1A1 219 QAIH NVVH AIIL HQQQ KQQQ
A1A6 219 QAIH NVVH AIIL HQQQ QKQQ
A1B3 219 QAIQ NVVH AIIL HQQQ KQQQ
A1B9 219 QAIH KVVH AIIL HQQQ KQQQ
A1B13 219 QAIH NVVH AIIL HQQQ QQQQ
A2B3 219 QAIH NVVH AIIL HQQH HHHQ
POOL 27
A3A1 219 QAIH NVVH AIIL HQQQ RQQQ
A3B1 219 QAIH NVVH AIIM HQQE QQQQ
A3B3 219 QAIH NVAH AIIM HQQQ QQQQ
A4A 219 QAIH NVVH AIIL HHHQ QQQQ
A1A1 227 AIIL HQQQ KQQQ QPSS QVSF
A1A6 227 AIIL HQQQ QKQQ QQPS SQFS
A1B2 227 AIIL HQQQ KQQQ QLSS QVSF
A1B10 227 AIIL HQQQ KQQQ PSSQ VSFQ
POOL 28
A1B13 227 AIIL HQQQ QQQQ EQKQ QLQQ
A2A1 227 AIIL HQQQ QQQQ QQQQ QPLS
A2B3 227 AIIL HQQH HHHQ QQQQ QQQQ
A2B4 227 AIIL HHHQ EQKQ QLQQ QQQQ
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A3B1 227 AIIM HQQE QQQQ LQQQ QQQQ
A3B3 227 AIIM HQQQ QQQQ EQKQ QLQQ
A4A 227 AIIL HHHQ QQQQ QPSS QVSY
POOL 29
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A1A6 235 KQQQ QPSS QFSF QQPL QQYP
A1B2 235 KQQQ QLSS QVSF QQPQ QQYP
A1B10 235 KQQQ PSSQ VSFQ QPQQ QYPL
A1B13 235 QQQQ EQKQ QLQQ QQQQ QQQL
A2B4 235 HHHQ EQKQ QLQQ QQQQ QQQL
A3A1 235 RQQQ PSSQ VSLQ QPQQ QYPS
A3B1 235 QQQQ LQQQ QQQQ LQQQ QQQQ
POOL 30
A4A 235 QQQQ QPSS QVSY QQPQ EQYP
A1B13 243 QLQQ QQQQ QQQL QQQQ QKQQ
A1B13 251 QQQL QQQQ QKQQ QQPS SQVS
A2A1 260 QQQQ QQQQ QPLS QVSF QQPQ
A2B1 260 QQQQ QQQQ QPLS QVCF QQSQ
A2B3 260 HHHQ QQQQ QQQQ QPLS QVSF
A3B1 260 QQQQ QQQQ QPSS QVSF QQPQ
A2A1 289 QPLS QVSF QQPQ QQYP SGQG
POOL 31
A2B1 289 QPLS QVCF QQSQ QQYP SGQG
A3B1 289 QPSS QVSF QQPQ QQYP SSQV

Gl6A 321 FQLA QGLG IIQP QQPA QLEG 508
GI1A 329 IIQP QQPA QLEA IRSL VLQT 509
GI3A 329 IIQP QQPA QLEV IRSL VLQT 510
POOL 66 .
GI3C 329 IIQP QQPA QLEV IRSS VLQT 511
GI5C 329 IIQP QQPA QYEV IRSL VLRT 512
Gl6A 329 IIQP QQPA QLEG IRSL VLKT 513
GI1A 337 QLEA IRSL VLQT LPTM CNVY 514
GI2A 337 QLEA IRSL VLQT LPSM CNVY 515
GI3A 337 QLEV IRSL VLQT LATM CNVY 516
GI3C 337 QLEV IRSS VLQT LATM CNVY 517
GI5A 337 QLEV IRSL VLGT LPTM CNVF 518
POOL 67 .
GI5C 337 QYEV IRSL VLRT LPNM CNVY 519
Gl6A 337 QLEG IRSL VLKT LPTM CNVY 520
GI1A 345 VLQT LPTM CNVY VPPE CSII 521
GI2A 345 VLQT LPSM CNVY VPPE CSIM 522
GI3A 345 VLQT LATM CNVY VPFY CSTI 523
GI5A 345 VLGT LPTM CNVF VPPE CSTT 524
GI5C 345 YLRT LPNM CNVY VRPD CSTI 525
Gl6A 345 VLKT LPTM CNVY VPPD CSTI 526
POOL 68 .
GI1A 353 CNVY VPPE CSII KAPF SSVV 527
GI2A 353 CNVY VPPE CSIM RAPF ASIV 528
GI3A 353 CNVY VPFY CSTI RAPF ASIV 529
GI5A 353 CNVF VPPE CSTT KAPF ASIV 530
GI5C 353 CNVY VRPD CSTI NAPF ASIV 531
Gl6A 353 CNVY VPPD CSTI NVPY ANID 532
GI1A 361 CSII KAPF SSVV AGIG GQ 533
GI2A 361 CSIM RAPF ASIV AGIG GQ 534
POOL 69 .
GI3A 361 CSTI RAPF ASIV AGIG GQYR 535
GI4 361 CSTI RAPF ASIV ASIG GQ 536
GI5A 361 CSTT KAPF ASIV ADIG GQ 537
GI5C 361 CSTI NAPF ASIV AGIS GQ 538
Gl6A 361 CSTI NVPY ANID AGIG GQ 539
GII 1 PQQP FPLQ PQQS FLWQ SQQP 540
GII 9 PQQS FLWQ SQQP FLQQ PQQP 541
GII 17 SQQP FLQQ PQQP SPQP QQVV 542
POOL 70 .
GII 25 PQQP SPQP QQVV QIIS PATP 543
GII 33 QQVV QIIS PATP TTIP SAGK 544
GII 41 PATP TTIP SAGK PTSA PFPQ 545
GII 49 SAGK PTSA PFPQ QQQQ HQQL 546
GII 57 PFPQ QQQQ HQQL AQQQ IPVV 547
GII 65 HQQL AQQQ IPVV QPSI LQQL 548
GII 73 IPVV QPSI LQQL NPCK VFLQ 549
GII 81 LQQL NPCK VFLQ QQCS PVAM 550
POOL 71 .
GII 89 VFLQ QQCS PVAM PQRL ARSQ 551
GII 97 PVAM PQRL ARSQ MLQQ SSCH 552
GII 105 ARSQ MLQQ SSCH VMQQ QCCQ 553
GII 113 SSCH VMQQ QCCQ QLPQ IPQQ 554
GII 121 QCCQ QLPQ IPQQ SRYQ AIRA 555
GII 127B PQIP QQSR YEAI RAII YSII 556
GII 129 IPQQ SRYQ AIRA IIYS IILQ 557
GII 137 AIRA IIYS IILQ EQQQ VQGS 558
POOL 72 .
GII 145 IILQ EQQQ VQGS IQSQ QQQP 559
GII 153 VQGS IQSQ QQQP QQLG QCVS 560
GII 161 QQQP QQLG QCVS QPQQ QSQQ 561
GII 169 QCVS QPQQ QSQQ QLGQ QPQQ 562
GII 177 QSQQ QLGQ QPQQ QQLA QGTF 563
GII 185 QPQQ QQLA QGTF LQPH QIAQ 564
POOL 73 .
GII 193 QGTF LQPH QIAQ LEVM TSIA 565
GII 201 QIAQ LEVM TSIA LRIL PTMC 566
GII 209 TSIA LRIL PTMC SVNV PLYR 567
GII 217 PTMC SVNV PLYR TTTS VPFG 568

A1A1 293 QVSF QQPL QQYP LGQG SFRP
A1A6 293 QFSF QQPL QQYP LGQG SFRP
A1B2 293 QVSF QQPQ QQYP LGQG SFRP
A2A1 293 QVSF QQPQ QQYP SGQG SFQP
A2B1 293 QVCF QQSQ QQYP SGQG SFQP
A2B3 293 QVSF QQPQ QQYP SGQG FFQP
POOL 32
A2B5 293 QVSF QQPQ QQYP SGQG FFQP
A3A1 293 QVSL QQPQ QQYP SGQG FFQP
A3B1 293 QVSF QQPQ QQYP SSQV SFQP
A3B2 293 QVSF QQPQ QQYP SSQG SFQP
A4A 293 QVSY QQPQ EQYP SGQV SFQS
A1A1 301 QQYP LGQG SFRP SQQN PQAQ
A1B2 301 QQYP LGQG SFRP SQQN SQAQ
A2A1 301 QQYP SGQG SFQP SQQN PQAQ
POOL 33
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A3A1 301 QQYP SGQG FFQP SQQN PQAQ
A3B1 301 QQYP SSQV SFQP SQLN PQAQ
A3B2 301 QQYP SSQG SFQP SQQN PQAQ
A4A 301 EQYP SGQV SFQS SQQN PQAQ
A1B1 309 SFRP SQQN PLAQ GSVQ PQQL
A1A1 309 SFRP SQQN PQAQ GSVQ PQQL
POOL 34
A1A3 309 SFRP SQQN PQTQ GSVQ PQQL
A1B2 309 SFRP SQQN SQAQ GSVQ PQQL
A1B3 309 SFRP SQQN PQDQ GSVQ PQQL
A1B4 309 SFRP SQQN PRAQ GSVQ PQQL
A2A1 309 SFQP SQQN PQAQ GSVQ PQQL
A2B3 309 FFQP SQQN PQAQ GSFQ PQQL
A2B5 309 FFQP FQQN PQAQ GSFQ PQQL
A3A1 309 FFQP SQQN PQAQ GSVQ PQQL
Pool 35
A3B1 309 SFQP SQLN PQAQ GSVQ PQQL
A3B1 309 SFQP SQLN PQAQ GSVQ PQQL
A3B2 309 SFQP SQQN PQAQ GSVQ PQQL
A4A 309 SFQS SQQN PQAQ GSVQ PQQL
A1A1 317 PQAQ GSVQ PQQL PQFE EIRN
A1A3 317 PQTQ GSVQ PQQL PQFE EIRN
A1A6 317 PQAQ GSVQ PQQL PQFE IRNL
A1B1 317 PLAQ GSVQ PQQL PQFE EIRN
POOL 36
A1B3 317 PQDQ GSVQ PQQL PQFE EIRN
A1B4 317 PRAQ GSVQ PQQL PQFE EIRN
A2B3 317 PQAQ GSFQ PQQL PQFE EIRN
A2B5 317 PQAQ GSFQ PQQL PQFE AIRN
A3B1 317 PQAQ GSVQ PQQL PQFA EIRN
A4A 317 PQAQ GSVQ PQQL PQFQ EIRN
Pool 37
A1A1 325 PQQL PQFE EIRN LALQ TLPA
A1A6 325 PQQL PQFE IRNL ALQT LPAM
A1B12 325 PQQL PQFE EIRN LARK
A2A1 325 PQQL PQFE EIRN LALE TLPA
A2B5 325 PQQL PQFE AIRN LALQ TLPA
A3B1 325 PQQL PQFA EIRN LALQ TLPA
A4A 325 PQQL PQFQ EIRN LALQ TLPA
A1A1 333 EIRN LALQ TLPA MCNV YIPP
POOL 38
A1A3 333 EIRN LALQ TLPS MCNV YIPP
A2A1 333 EIRN LALE TLPA MCNV YIPP
A3A1 333 EIRN LALQ TLPR MCNV YIPP
A1A1 341 TLPA MCNV YIPP YCTI APFG
A1A3 341 TLPS MCNV YIPP YCTI APFG
A1B1 341 TLPA MCNV YIPP YCTI VPFG
A1B4 341 TLPA MCNV YIPP YCAM APFG
A1B9 341 TLPA MCNV YIPP YCTI TPFG
Pool 39
A2A1 341 TLPA MCNV YIPP YCTI APVG

GII 225 PLYR TTTS VPFG VGTG VGAY
GIII 1A 1 TITR TFPI PTIS SNNN HHFR
GIII 1A 9 PTIS SNNN HHFR SNSN HHFH
GIII 1A 17 HHFR SNSN HHFH SNNN QFYR
POOL 74
GIII 1A 25 HHFH SNNN QFYR NNNS PGHN
GIII 1A 33 QFYR NNNS PGHN NPLN NNNS
GIII 1A 41 PGHN NPLN NNNS PNNN SPSN
GIII 1A 49 NNNS PNNN SPSN HHNN SPNN
GIII 1A 57 SPSN HHNN SPNN NFQY HTHP
GIII 1A 65 SPNN NFQY HTHP SNHK NLPH
GIII 1A 73 HTHP SNHK NLPH TNNI QQQQ
GIII 1A 81 NLPH TNNI QQQQ PPFS QQQQ
POOL 75
GIII 1A 89 QQQQ PPFS QQQQ PPFS QQQQ
GIII 1A 97 QQQQ PPFS QQQQ PVLP QQSP
GIII 1A 105 QQQQ PVLP QQSP FSQQ QQLV
GIII 1A 113 QQSP FSQQ QQLV LPPQ QQQQ
GIII 1A 121 QQLV LPPQ QQQQ QLVQ QQIP
GIII 1A 129 QQQQ QLVQ QQIP IVQP SVLQ
GIII 1A 137 QQIP IVQP SVLQ QLNP CKVF
GIII 1A 145 SVLQ QLNP CKVF LQQQ CSPV
POOL 76
GIII 1A 153 CKVF LQQQ CSPV AMPQ RLAR
GIII 1A 161 CSPV AMPQ RLAR SQMW QQSS
GIII 1A 169 RLAR SQMW QQSS CHVM QQQC
GIII 1A 177 QQSS CHVM QQQC CQQL QQIP
GIII 1A 185 QQQC CQQL QQIP EQSH YEAI
GIII 1A 193 QQIP EQSR YEAI RAII YSII
GIII 1A 201 YEAI RAII YSII LQEQ QQGF
GIII 1A 209 YSII LQEQ QQGF VQPQ QQQP
POOL 77
GIII 1A 217 QQGF VQPQ QQQP QQSG QGVS
GIII 1A 225 QQQP QQSG QGVS QSQQ QSQQ
GIII 1A 233 QGVS QSQQ QSQQ QLGQ CSFQ
GIII 1A 241 QSQQ QLGQ CSFQ QPQQ QLGQ
GIII 1A 249 CSFQ QPQQ QLGQ QPQQ QQQQ
GIII 1A 257 QLGQ QPQQ QQQQ QVLQ GTFL
GIII 1A 263 QQQQ QVLQ GTFL QPHQ IAHL
GIII 1A 271 GTFL QPHQ IAHL EAVT SIAL
POOL 78
GIII 1A 279 IAHL EAVT SIAL RTLP TMCS
GIII 1A 287 SIAL RTLP TMCS VNVP LYSA
GIII 1A 295 TMCS VNVP LYSA TTSV PFGV
GIII 1A 303 LYSA TTSV PFGV GTGV GAY
GIII 1B 26 SCIS GLER PWQQ QPLP PQQS
GIII 1B 34 PWQQ QPLP PQQS FSQQ PPFS
GIII 1B 42 PQQS FSQQ PPFS QQQQ QPLP
GIII 1B 50 PPFS QQQQ QPLP QQPS FSQQ
Pool 79
GIII 1B 58 QPLP QQPS FSQQ QPPF SQQQ
GIII 1B 66 FSQQ QPPF SQQQ PILS QQPP
GIII 1B 74 SQQQ PILS QQPP FSQQ QQPV
O 1A 17 ATAA RELN PSNK ELQS PQQS
O 1A 25 PSNK ELQS PQQS FSYQ QQPF
O 1A 33 PQQS FSYQ QQPF PQQP YPQQ
O 1A 41 QQPF PQQP YPQQ PYPS QQPY
O 1A 49 YPQQ PYPS QQPY PSQQ PFPT
POOL 80
O 1A 57 QQPY PSQQ PFPT PQQQ FPEQ
O 1A 65 PFPT PQQQ FPEQ SQQP FTQP
O 1A 73 FPEQ SQQP FTQP QQPT PIQP
O 1A 81 FTQP QQPT PIQP QQPF PQQP
O 1A 89 PIQP QQPF PQQP QQPQ QPFP
O 1A 97 PQQP QQPQ QPFP QPQQ PFPW
O 1A 105 QPFP QPQQ PFPW QPQQ PFPQ
O 1A 113 PFPW QPQQ PFPQ TQQS FPLQ
POOL 81
O 1A 121 PFPQ TQQS FPLQ PQQP FPQQ

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A2B2 341 TLPA MCNV YIPP YC**ST** TIAP 300
A3A1 341 TLP**R** MCNV YIPP YC**ST** TIAP 301
A3A2 341 TLPR MCNV YIPP YCST T**T**AP 302
A3B1 341 TLPA MCNV YIPP **HCST** TIAP 303
A1A1 349 YIPP YCTI APFG IFGT NYR 304
A1B1 349 YIPP YCTI VPFG IFGT NYR 305
A1B4 349 YIPP YC**A**M APFG IFGT NYR 306

Pool 40

A1B5 349 YIPP YCT**M** APFG IFGT NYR 307
A1B9 349 YIPP YCTI **T**PFG IFGT N 308
A2A1 349 YIPP YCTI APVG IFGT NYR 309
A2B2 349 YIPP YC**ST** TIAP VGIF GTN 310
A3A2 349 YIPP YCST T**T**AP FGIF GTN 311
A3B1 349 YIPP **HCST** TIAP FGIF GTN 312
A3B3 349 YIPP HCST TIAP FGI**S** GTN 313
A4D 350 IPPY CSTT IAPF GIFG TNYR 314

Pool 41

GI1A 17 GTAN M**Q**VD PSS**Q** VQW**P** QQ**P** 315
GI2A 17 GTAN I**Q**VD PSG**Q** VQW**L** QQ**L** 316
GI3A 17 ATAN M**Q**VD PSG**Q** VPW**P** QQ**P** 317
GI3B 19 MN IQVD PSGQ VPW**P** QQQ**P** FP 318
GI4 17 ATAN MQAD PSG**Q** VQW**P** QQQ**P** 319
GI5A 17 **T**TAN IQVD PSGQ VQW**P** QQQQ 320
GI5C 17 ATAN MQVD PSGQ VQWP QQQ**P** 321
GI7 20 QIVF PSGQ VQW**P** QQQQ PFP 322

Pool 42

GI1A 25 PS**S**Q VQW**P** QQQ**P** V**P**Q**P** H**Q**P**F** 323
GI2A 25 PS**G**Q VQW**L** QQQ**L** V**P**Q**L** Q**Q**P**L** 324
GI3A 25 PS**G**Q VPW**P** QQQ**P** F**P**Q**P** H**Q**P**F** 325
GI4 25 PS**G**Q VQW**P** QQQ**P** F**L**Q**P** H**Q**P**F** 326
GI5A 25 PSGQ VQWP QQQQ PFPQ PQQP 327
GI5C 25 PSGQ VQWP QQQP FRQP QQPF 328
GI6A 25 PSGQ VQWP QQQP FPQP QQPF 329
GI1A 33 **Q**Q**Q**P V**P**Q**P** H**Q**P**F** S**Q**Q**P** Q**Q**TF 330

O 1A 129 FPLQ PQQP FPQQ PQQP FPQP 630
O 1A 137 FPQQ PQQP FPQP QLPF PQQS 631
O 1A 145 FPQP QLPF PQQS EQH PQQL 632
O 1A 153 PQQS EQH PQQL QQPF PLQP 633
O 1A 161 PQQL QQPF PLQP QQPF PQQP 634
O 1A 169 PLQP QQPF PQQP QQPF PQPQ 635
O 1A 177 PQQP QQPF PQPQ QPIP VQPQ 636

Pool 82

O 1A 185 PQPQ QPIP VQPQ QSFP QQSQ 637
O 1A 193 VQPQ QSFP QQSQ QSQQ PFAQ 638
O 1A 201 QQSQ QSQQ PFAQ PQQL FPEL 639
O 1A 209 PFAQ PQQL FPEL QQPF PQQP 640
O 1A 217 FPEL QQPF PQQP QQPF PLQP 641
O 1A 225 PQQP QQPF PLQP QQPF PQQP 642
O 1A 233 PLQP QQPF PQQP QQPF PQQP 643
O 1A 241 PQQP QQPF PQQP QQSF PQQP 644

POOL 83

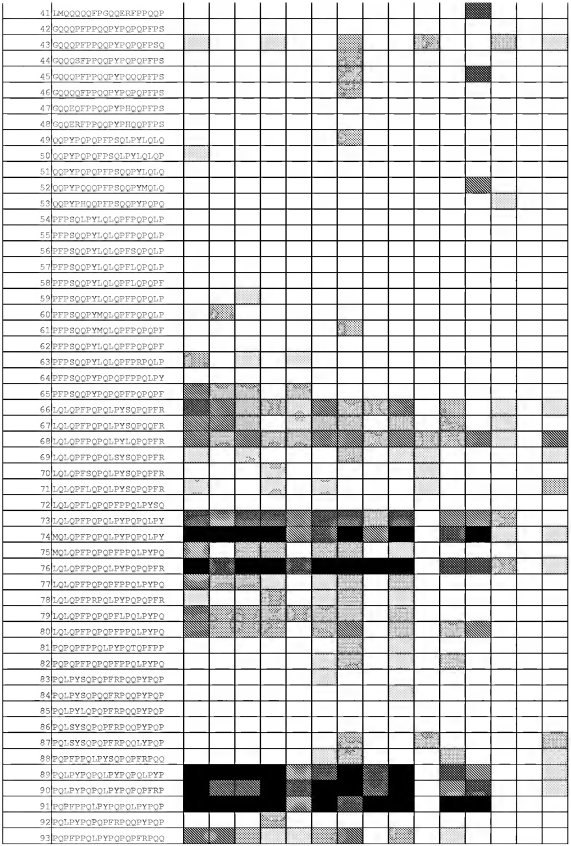
O 1A 249 PQQP QQSF PQQP QQPY PQQQ 645
O 1A 257 PQQP QQPY PQQQ PYGS SLTS 646
O 1A 265 PQQQ PYGS SLTS IGGQ 647
O 1B I ARQL NPSD QELQ SPQQ LYPQ 648
O 1B 9 QELQ SPQQ LYPQ QPYP QQPY 649
O 1C I SRLL SPRG KELH TPQE QFPQ 650
O 1C 9 KELH TPQE QFPQ QQQF PQPQ 651
O 1C 17 QFPQ QQQF PQPQ QFPQ 652

*Position of N-terminal residue in α-, γ1-, γ2-, γ3-, or ω consensus sequence

Table 24. 652 synthetic peptides and ELISpot analysis with patients and gluten challenge (Peptide Numbers 1-652 correspond to SEQ ID NO. 107 through SEQ ID NO:758, respectively)

COELIAC SUBJECT	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28
HLA-DQ	22	22	22	22	22	22	2X	2X	2X	2X	28	28	2X	2X	2X
ANTIGEN CHALLENGE	WHEAT												RYE		
DURATION OF CHALLENGE (DAYS)	3	3	6	3	6	3	3	3	3	0.5	3	1	3	3	3
DOMINANT PEPTIDE ELISPOT SFC	203	46	96	195	114	136	29	57	129	259	50	18	163	52	229
BLANK ELISPOT SFC	1	2	4	0.5	3	1	1	4	3	2	1	2	1	2	6

Peptide	Sequence
1	AVRFPVPQLQPQNPSQQLPQ
2	MVRVPVPQLQPQNPSQQQPQ
3	MVRVPVPQLQPQNPSQQHPQ
4	MVRVPMPQLQPQDPSQQQPQ
5	MVRVTVPQLQPQNPSQQQPQ
6	AVRVSVPQLQPQNPSQQQPQ
7	AVRVPVPQLQPQNPSQQQPQ
8	AVRWPVPQLQPQNPSQQQPQ
9	AVRVPVPQLQLQNPSQQQPQ
10	MVRVPVPQLQLQNPSQQQPQ
11	AVRVPVPQPQPQNPSQPQPQ
12	AVRVPVPQLQPKNPSQQQPQ
13	LQPQNPSQQLPQEQVPLVQQ
14	LQPQNPSQQQPQEQVPLVQQ
15	LQPQNPSQQHPQEQVPLVQQ
16	LQPQDPSQQQPQEQVPLVQQ
17	LQPQNPSQQQPQKQVPLVQQ
18	LQLQNPSQQQPQEQVPLVQE
19	TQLQNPSQQQPQEQVPLVQE
20	PQPQNPSQPQPQGQVPLVQQ
21	PQPQNPSQPQPQRQVPLVQQ
22	LQPKNPSQQQPQEQVPLVQQ
23	LQPQNPSQQQPQEQVPLMQQ
24	QLPQEQVPLVQQQQFLQQQQ
25	QHPQEQVPLVQQQQFLQQQQ
26	QQPQEQVPLVQQQQFLQQQQ
27	QQPQEQVPLVQQQQFLQQQQ
28	QQPQEQVPLVQQQQFPQQQQ
29	QQPQKQVPLVQQQQFPQQQQ
30	QQPQEQVPLVQEQQFQQQQQ
31	PQPQSQVPLVQQQQFPQQQQ
32	PQPQRQVPLVQQQQFPQQQQ
33	QQPQEQVPLMQQQQQFPQQQ
34	LVQQQQFLVQQQPFPPQQPY
35	LVQQQQFLVQQQSFPPQQPY
36	LVQQQQFLVQQQPFPPQQPY
37	LVQQQQFPQQQPFPPQQPY
38	LVQKQQFGQQQPFPPQQPY
39	LVQQQQFPSQQQQFPPQQPY
40	LMQQQQQFPGQQKQFPPQQP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50 GQPYPQPQFPSQLPYLQLQP
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79 LQLQPFPQPQPFLPQLPYPQ
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84 PQLPYSQPQQFPRQQPYPQP
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86 PQLSYSQPQPFPRQQPYPQP
87 PQLSYSQPQPFPRQQLYPQP
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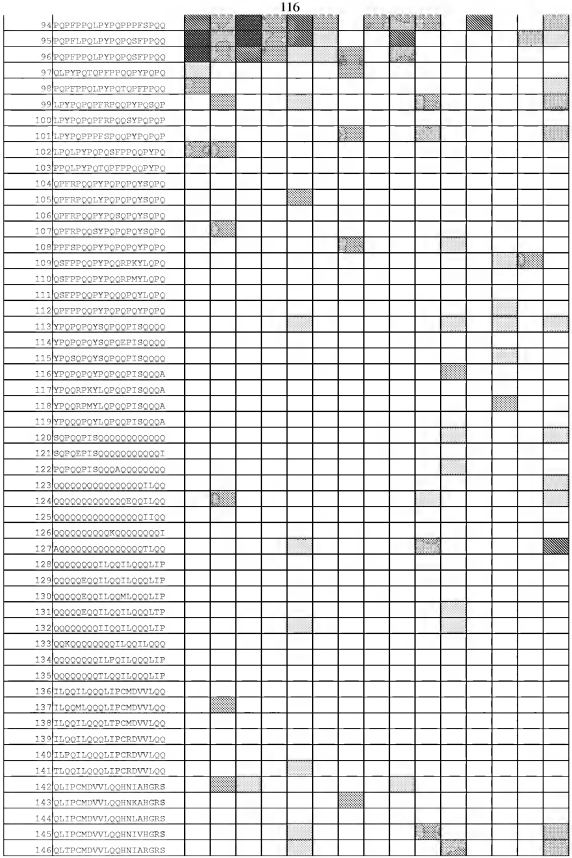
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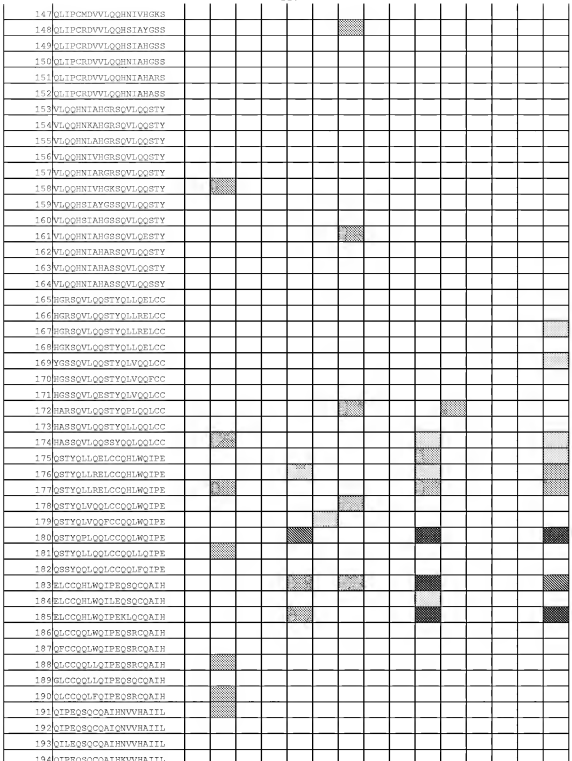
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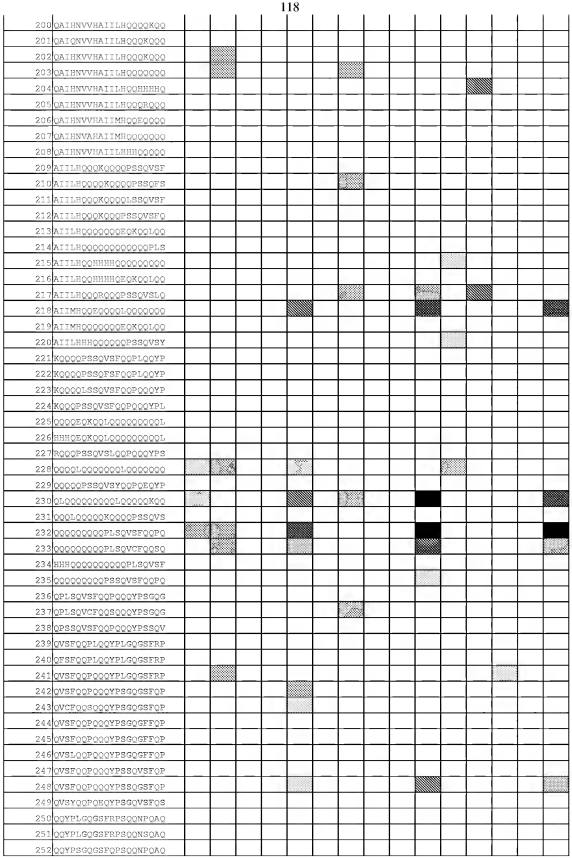
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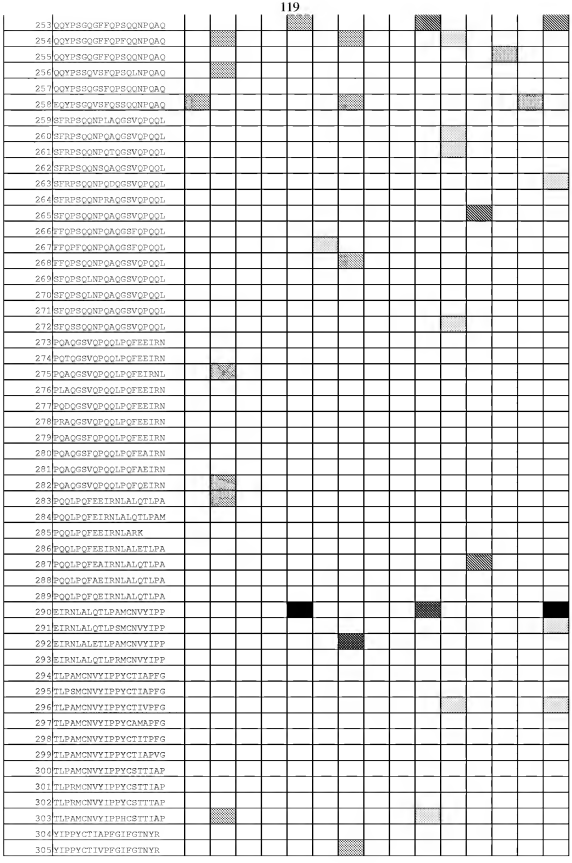
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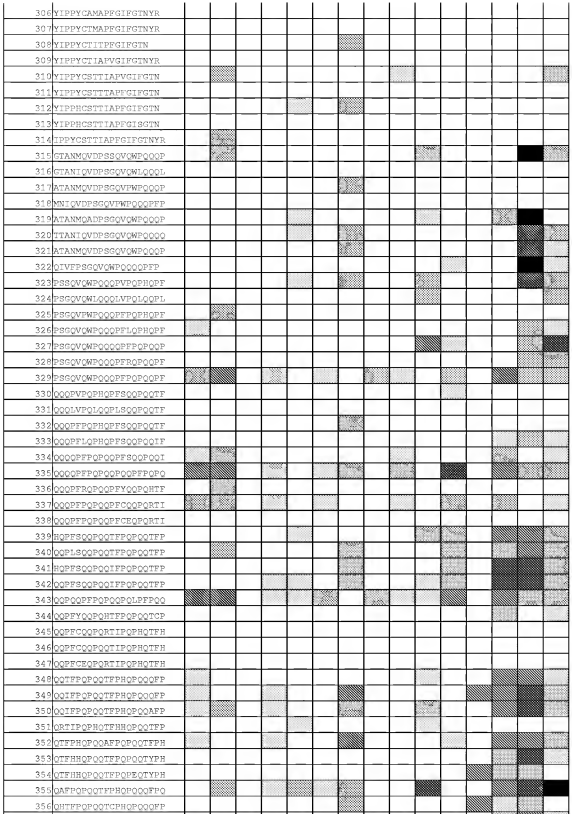
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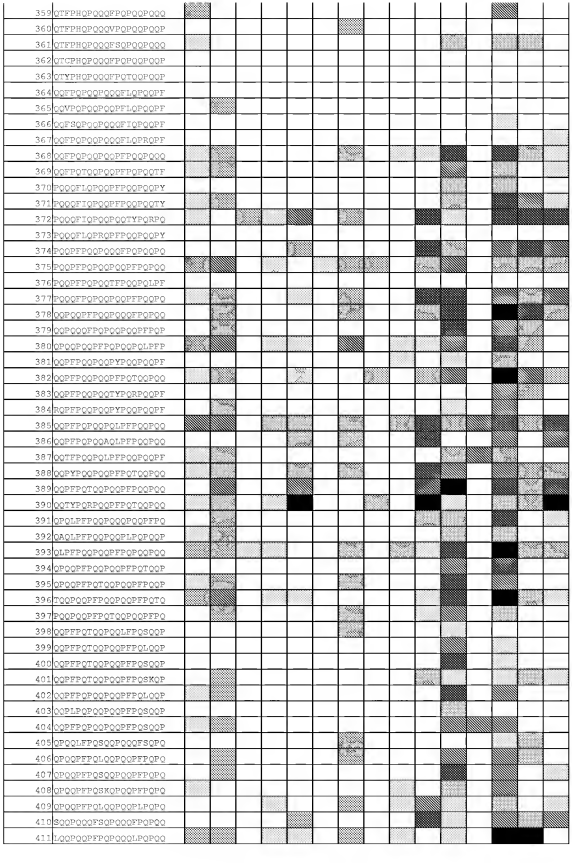
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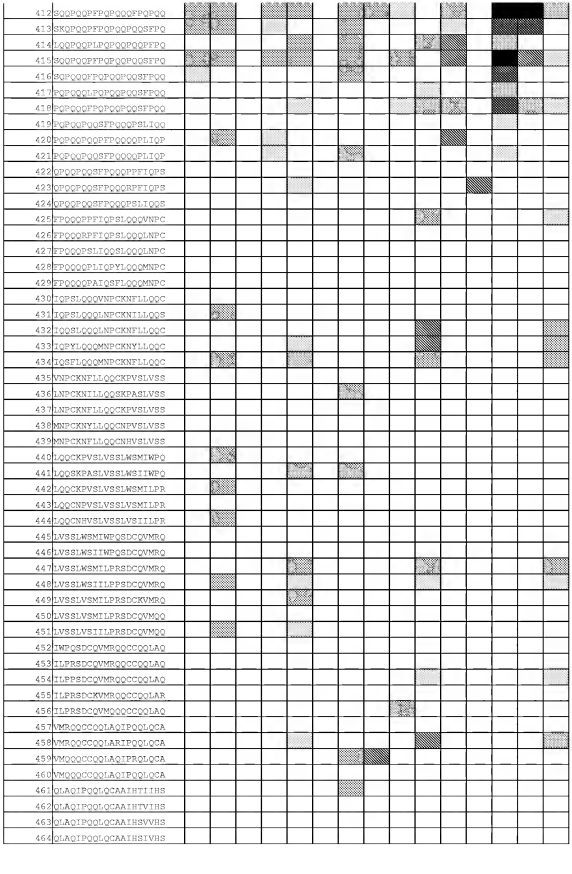
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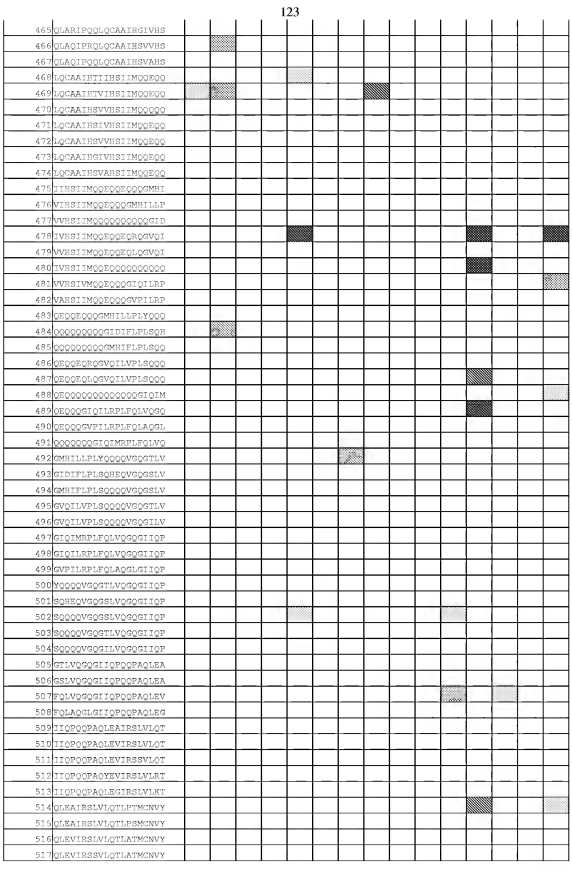
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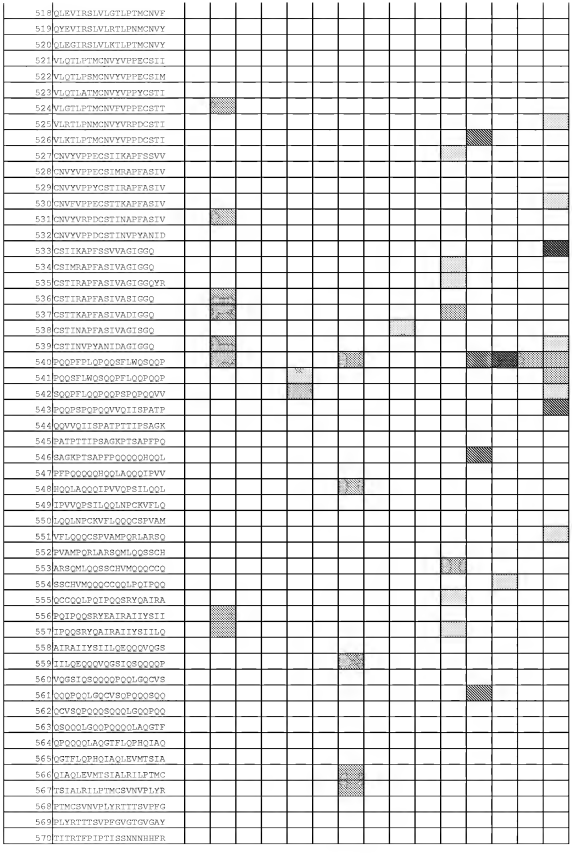
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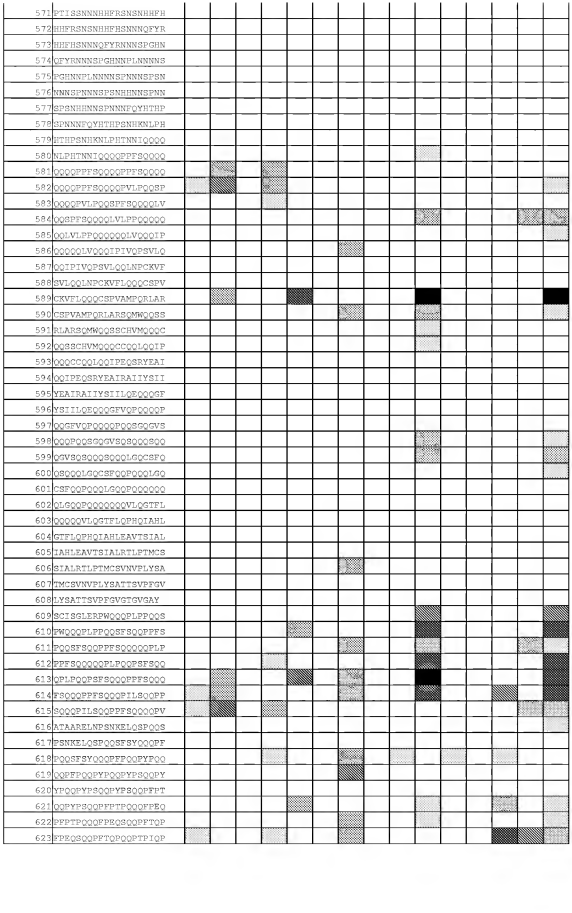
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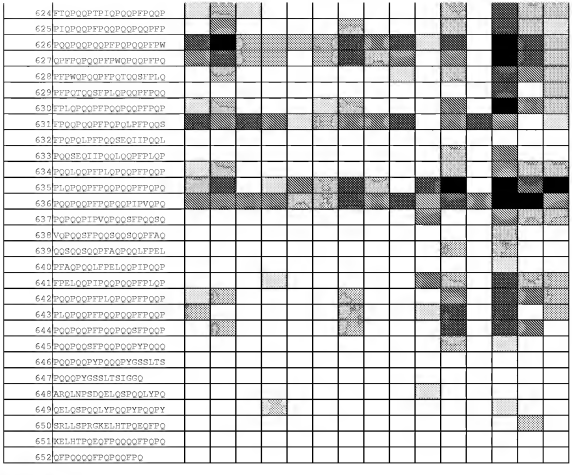

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